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ALLEN'S  
COMMERCIAL ORGANIC ANALYSIS

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VOLUME IX

# CONTRIBUTORS

## TO VOLUME IX

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# ALLEN'S COMMERCIAL ORGANIC ANALYSIS

A TREATISE ON  
THE PROPERTIES, MODES OF ANALYSIS, AND PROXIMATE  
ANALYTICAL EXAMINATION OF THE VARIOUS  
ORGANIC CHEMICALS AND PRODUCTS  
EMPLOYED IN THE ARTS, MANU-  
FACTURES, MEDICINE, Etc.

WITH CONCISE METHODS FOR  
THE DETECTION AND ESTIMATION OF THEIR IMPURITIES,  
ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

## VOLUME IX

The Proteins of Plants, The Proteins of Milk, Milk, Milk Products  
Meat and Meat Products

BY THE EDITOR AND THE FOLLOWING CONTRIBUTORS

D. JORDAN LLOYD, G. D. ELSDON, H. LEFFMANN and  
JOHN GOLDING, E. R. BOLTON, C. ROBERT MOULTON

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## PREFACE

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It was hoped that the present edition of Allen's *Commercial Organic Analysis* could be completed in nine volumes. When, however, it was realised that the present volume would be disproportionately large and unwieldy in comparison with the earlier ones, it was decided to issue a tenth volume, and unfortunately this course has necessitated the further division of the subject of proteins.

Since the revision of the articles in this volume was begun, Dr. Henry Leffmann, who was responsible for the section on milk, has died. He had been in poor health for some time, and it was at his special request that Captain J. Golding undertook the revision of that section. Unfortunately, Dr. L. L. Van Slyke was unable to revise his former contribution on the proteins of milk, and the work has therefore been undertaken by Mr. G. D. Elsdon. The section on plant proteins has been rewritten by Dr. D. Jordan Lloyd, and that on meat and meat products, originally by Mr. W. D. Richardson, has been replaced by what may be described as a comprehensive treatise by Dr. C. R. Moulton. The only contributor whose name appears in both editions is Mr. E. R. Bolton, who has revised the article on milk products which was written by him in collaboration with Mr. C. Revis in the last edition.

C. AINSWORTH MITCHELL.



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# THE PROTEINS OF PLANTS

BY D. JORDAN LLOYD, M. A., D. Sc., F. I. C.

The proteins of plants have the same general chemical properties as those of animals. They contain carbon, hydrogen, nitrogen, sulphur and sometimes phosphorus, and yield on hydrolysis a mixture of the same amino acids as are obtained from animal proteins. The most notable differences in the hydrolytic products of the two classes are that many plant proteins yield much larger quantities of glutaminic and aspartic acids than are obtained from animal proteins, and that they also commonly give a high yield of arginine and a low yield of lysine.

**Classification of Vegetable Proteins.**—The vegetable proteins can be classified according to their solubilities in four different groups—albumins, globulins, prolamins (gliadins) or alcohol-soluble proteins, and glutelins or alkali-soluble proteins. (For the definition of these classes, Vol. VIII, p. 642.) Vegetable albumins and globulins do not form two well-defined groups; the globulins, for instance, are precipitated by various degrees of saturation with ammonium sulphate, some requiring full saturation. A number of the vegetable globulins have been obtained in the crystalline state; of these, edestin from hemp seed is probably the best known. According to their commercial value as human and animal foods, vegetable proteins may be considered conveniently under five headings, namely, (1) proteins from cereal seeds, (2) from leguminous seeds, (3) from nuts, (4) from oil seeds, (5) from green leaves, fruits, roots and tubers.

The cereal seeds are the only group which contains prolamins and glutelins in addition to albumins and globulins. Leguminous seeds, nuts, oil seeds, fruits, roots and tubers contain albumins and globulins only. Green leaves contain heat-coagulable proteins allied to albumins and globulins and somewhat resembling the glutelins as regards their solubility in alkaline solutions, but difficult

to assign to any recognised group. These different classes of vegetable proteins will be considered separately below. All plant seeds contain, of course, the reserve proteins of the endosperm as well as the proteins from the tissues of the embryo. Fruits and green leaves contain only tissue proteins.

The great majority of plant proteins are edible but it is interesting to notice that a few of them are poisonous, the best known being ricin from the castor-oil bean. An account of the toxic proteins is given by Osborne in his monograph on "*The Vegetable Proteins.*"

**Protein Content of Cereals.**—The main commercial value of the plant proteins is as food materials. In cereals, 15% of the weight of the seed may be protein, and in the pulses or leguminous seeds the protein content may rise as high as 40%. In nuts and oil seeds, it may vary from about 6 to 20%; with green plants and vegetables, fruits, roots and tubers, it is frequently less than 1. A knowledge of the protein content of any vegetable product is, therefore, of importance. This is invariably obtained by a determination of the *total nitrogen* by Kjeldahl's method (see Vol. VIII, p. 658) and multiplication of the nitrogen figure by a factor. This factor has been the subject of much discussion (see Kent-Jones, 1930, *Recent Advances in Analytical Chemistry*, Chap. VI, Cereals, p. 217; also Breese-Jones, *Cer. Chem.* 1926, 3, 194). Obviously, it will be liable to differ with every protein and even in such a limited group as the cereals it has been shown to vary from 5.7 for wheat to 6.39 for maize. For general use, 5.7 is taken as the conversion factor for wheat proteins and 6.25 for the other plant proteins. This method of estimating protein content as nitrogen multiplied by a factor, although justifiable for seeds and seed products, is extremely misleading for the green parts of plants, since less than half the nitrogen in these has been shown to be present as protein. The protein content of cereals, pulses, nuts, oil seeds and the so-called protein content for fresh fruits and vegetables are given in Tables 3 and 5 to 8, respectively, together with figures for water, oil, carbohydrate and ash for the first three classes mentioned. For further analytical figures, the reader is referred to the works of König, Tibbles, Schall and Heisler. Smetham (*The Analyst*, 1914, 39, 481; *J. Soc. Chem. Ind.* 1910, p. 1107, 1914, 39); and Bryant and Atwater (*Bull. U. S. Bureau Agric.*, Nos. 28 and 132) also give analyses of a large number of food materials.

**Food Value of Proteins.**—It is, however, now being realised that the value of a foodstuff cannot be calculated directly from its protein content, but depends very largely upon the amino acid constitution of the protein present. In order to obtain light on the constitution of a protein, it must first be extracted from the vegetable matter and obtained in as pure a form as possible. For the general methods of extracting and separating proteins, see Vol. VIII pp. 652–657 and 662–670. The proteins of seeds are, in general, readily extracted. The seeds should be passed through a small grinding mill and the finely ground material shaken with twice its weight of a 10% sodium chloride solution. This treatment dissolves out albumins and globulins, which are the only classes of proteins found in seeds, with the exception of the cereal seeds. The latter also contain prolamins (soluble in aqueous alcohol) and glutelins (soluble in dilute alkali). The special methods recommended for extracting and separating these are dealt with below in the section on Cereal Proteins. The proteins of the green parts of plants are only extracted with difficulty after grinding up the tissues to break up the cellulose walls of the cells. For details of method, reference should be made to the papers of Chibnall and Nolan and of McKee and Smith, quoted in Table 2.

The vegetable protein having been obtained in pure form and analysed for total nitrogen, it can be hydrolysed and separated into its different amino acid constituents. This is a very tedious business, but since most of the biologically important amino acids occur in the basic group (di-amino acids), valuable information on the constitution of any protein can be obtained by the determination of the "Hausmann numbers" by means of a Van Slyke analysis (see Vol. VIII, p. 693, see also Van Slyke, *J. Biol. Chem.*, 1911, 9, 185).

**Hausmann Numbers.**—The Hausmann numbers classify the nitrogen present in a protein as (1) ammonia nitrogen, (2) humin nitrogen, (3) basic or di-amino nitrogen, (4) non-basic nitrogen. This last class is frequently subdivided into mono-amino nitrogen and non-amino nitrogen. A full Van Slyke analysis sub-divides the basic or di-amino fraction into lysine nitrogen, arginine, cystine and histidine nitrogen. In this analysis cystine is determined by calculation from the total sulphur content. This is a proceeding of dubious value, since it is now known that cystine is not the only sulphur-containing amino acid in proteins, though up to the present,

it is the only one which has been isolated from vegetable proteins (see Jordan Lloyd, *Chemistry of the Proteins*, p. 78, for further references on this point).

**Sulphur in Proteins.**—The determination of total sulphur in proteins or vegetable foods is generally effected by the oxidation of the material and the conversion of the sulphur present into a soluble sulphate which can then be separated as the insoluble barium sulphate. This oxidation may be carried out by Carius' method of heating with fuming nitric acid to about  $200^{\circ}$  in a sealed tube. Another method is Benedict's method as modified by Wolf and Osterberg (see Vol. VIII, p. 692). This method consists in boiling the protein with fuming nitric acid until it has passed into solution and completing the oxidation by the addition of copper nitrate and potassium chlorate, the whole solution being evaporated to dryness and the residue finally ignited at a red heat, for which purpose Givens (*J. Biol. Chem.*, 1917, **29**, 15) recommends an electric hot plate. The method has been criticised, since considerable splashing occurs in the early stages of the ignition, leading to loss of material. Robison (*Biochem. J.*, 1922, **16**, 134) has shown that by avoiding the use of chlorate or alkali salts the temperature of the final ignition can be greatly reduced and splashing avoided. His method (worked out originally for urine) can be used for any organic compound containing sulphur which can be brought into solution. He points out that the use of gas for heating must be scrupulously avoided. *Robison's method* is as follows:

"The oxidising reagent finally adopted has the following composition. Copper nitrate (crystals), 40 grm.; copper chloride (crystals) 15 grm.; water to 100 c.c. 2.5 c.c. of this solution are added to 10 c.c. of the urine (or other solution under examination) in a 4-inch porcelain basin and evaporated to dryness on a water bath or electric hot plate. The oxidation can be started on the hot plate or over a very small spirit flame. It proceeds rapidly but smoothly, leaving a coherent residue which frequently swells up. The dish is then heated over a broad spirit flame for 20 minutes. A spirit stove of the common kind is suitable, but a sound tin, half filled with methylated spirit answers very well. A better flame is obtained if a number of holes are punched half way up the tin. The residue is dissolved in 10 c.c. of 2 *N* hydrochloric acid and diluted with 300 c.c. of distilled water. The sulphate is precipitated

in the boiling solution with 10 c.c. of a 5% solution of barium chloride dropped in very slowly by means of a dropping tube. The precipitate is allowed to stand overnight before being filtered."

A number of analyses showing the distribution of nitrogen in plant proteins and the percentage of nitrogen and sulphur are given in Table 1. The former lies between 15 and 18% and the latter between a half and one and a half per cent.

The Hausmann numbers and the sulphur content of the protein are often of considerable use in forming an opinion as to its value as a food. A high figure for basic nitrogen can be taken as a first indication that the protein in question is likely to have a high food value. It will be noticed that the prolamins as a class are low in basic nitrogen. The poorest is zein, the prolamins of maize, which forms half the protein present in the maize seed. The glutelins seem to contain considerable quantities of basic nitrogen and the albumins and globulins of plants are not inferior in this respect to those of animals.

### SEPARATION OF AMINO ACIDS

It is now being realised, however, that the biological value of the food protein can only be determined after a full knowledge has been obtained of its constitution. The final stage of the protein analysis is, therefore, the separation and determination of all the amino acids present in the protein. For a complete analysis, three general methods are available, Dakin's butyl-alcohol method, Foreman's lead-salt method and Kingston and Schryver's carbamate method (see Vol. VIII, p. 626 *et. seq.*). A description of these three general methods and of a number of special methods for particular units are given by Jordan Lloyd (*The Chemistry of the Proteins*). Town (*Biochem. J.*, 1928, **22**, 1083) has recently described a new method of separation based on the differential solubilities of the copper salts which has also been used successfully by Brazier (*Biochem. J.* 1930, **24**, 1188). The special units in the protein molecule that have now been shown to be essential in the mammalian diet are tryptophane, cystine, lysine, and arginine and possibly histidine, tyrosine, phenylalanine and proline. For a discussion of the evidence on this point, see Jordan Lloyd, "*Chemistry of the Proteins*" and McCollum and Simmonds "*The Newer Knowledge of Nutrition*." It will be noticed that the percentages of lysine, histidine and



arginine are obtained from the Van Slyke analysis. An indication of the amount of tryptophane present can be obtained from the value of the humin nitrogen and an indication of cystine from the sulphur determination. These two last figures, however, are very unsatisfactory, since the formation of humin is influenced by the presence of carbohydrates and cystine is now known to be not the only sulphur-containing amino acid. Certain special colorimetric methods are now available for determining the amount of these units in a protein.

**Tryptophane** can be determined by the method of May and Rose (see Vol. VIII, p. 698).

**Cystine** can be determined by the method of Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421) which has recently been improved by Folin and Marenzi (*J. Biol. Chem.*, 1929, **83**, 89). This method involves the use of the "uric acid reagent" of Folin and Denis (*J. Biol. Chem.*, 1912, **12**, 239). The reagent was originally prepared as follows:

"To 750 grm. of water add 100 grm. of sodium tungstate and 80 c.c. of 85% phosphoric acid ( $\text{H}_3\text{PO}_4$ ). Boil gently for 2 hours, using a reflux condenser to prevent undue concentration. Cool and dilute to 1 litre."

Since in a number of cases difficulties have arisen through commercial brands of sodium tungstate not being free from molybdate, Folin and Marenzi have now described a method for preparing the uric acid reagent so that it is freed with certainty from phenolic bodies and molybdates. These instructions are given in full below:—

"Transfer 100 grm. of sodium tungstate and 200 c.c. of water to a 500 c.c. Florence flask. Shake until the tungstate is dissolved. Add slowly, with shaking and cooling, 20 c.c. of 85% phosphoric acid. The solution must not be allowed to become warm from the heat of the reaction with the phosphoric acid. Pass  $\text{H}_2\text{S}$  into the phosphotungstate solution at a very moderate rate for 20 minutes. At the end of the first 3 or 4 minutes, add gradually and slowly another 10 c.c. of 85% phosphoric acid without interrupting the  $\text{H}_2\text{S}$  current. It would be simpler to add all of the phosphoric acid (30 c.c.) before beginning the  $\text{H}_2\text{S}$  treatment, but by adding the phosphoric acid as described, one obtains the molybdenum sulphide in a somewhat less finely divided condition, so that it can be removed more easily by filtration. Incidentally, it may be

remarked that the 30 c.c. of phosphoric acid should be just sufficient to render the solution slightly acid to Congo red paper. At the end of 20 minutes, filter the solution through a good grade of quantitative filter paper. It is advisable to collect the first 40 c.c. of filtrate in a 50 c.c. cylinder, because the first portion may be a little turbid, and it may need to pass through the filter a second time.

"If the conditions have been right, the filtrate should be clear, and it will have a greenish colour, because a little blue is produced by reduction of any uric acid reagent that may have formed, while the soluble sulfomolybdates are red.

"Transfer the filtrate to a separatory funnel (capacity 1 liter) and add, with shaking, 300 c.c. (1.5 volumes) of alcohol. The mixture separates at once into a reddish or slightly greenish supernatant solution, and a bluish, very heavy solution at the bottom. The latter contains all of the phosphotungstic acid in a supersaturated solution, and it is best to withdraw it rather soon into a weighed 500 c.c. Florence flask. If left too long in the separatory funnel, it sometimes forms crystal deposits which block the exit through the stop-cock.

"In so far as any insoluble molybdenum sulphide happens to be present, this will be floating between the two layers of liquid in the separatory funnel, and these solid aggregates must not be allowed to pass through the stop-cock and into the phosphotungstic acid solution. The mixture remaining in the separatory funnel is discarded. It contains not only the sulphomolybdates, and the greater part of the surplus  $H_2S$ , but probably also various other impurities.

"Add water to the concentrated phosphotungstic acid in the 500 c.c. flask until the weight of the contents amounts to 300 grm. Boil the solution over a micro burner for a few minutes, until a paper moistened with lead acetate solution shows that the  $H_2S$  has been removed. Then, but not until then, cut down the flame, and add 20 c.c. of 85% phosphoric acid. It is only with the addition of this last quantity of phosphoric acid that the optimum conditions are obtained for transforming the ordinary (1:24) phosphotungstic acid into the active (1:18) phosphotungstic acid, that is to say, into the uric acid reagent.

"Insert a 10 cm. funnel into the 500 c.c. flask to hold a 200 c.c. flask filled with cold water, and boil gently for 1 hour. At the end

of this time, the reaction is finished. Cut down the flame, remove the condenser (funnel and flask), filter and add to the filtrate a few drops of bromine, and boil, to remove the blue colour of the solution. When the blue colour is gone, boil rapidly for a few minutes, to remove the bromine, then cover the mouth of the flask with a beaker and cool under running water.

"Transfer 25 grm. of lithium carbonate to a litre beaker, add first 50 c.c. of phosphoric acid, then add slowly 250 c.c. of water and boil, to remove the  $\text{CO}_2$ . Cool the resulting lithium phosphate solution; and add it to the concentrated uric acid reagent in the 500 c.c. flask and dilute to 1 litre."

The uric acid reagent should be added to the solution under examination in the presence of an acid, but the mixture must be made alkaline before the colour will develop. Excess of alkali leads to fading of the colour; nitrates must be absent. Folin and Looney give the procedure for the determination of cystine is as follows:

"From 1-5 grm. of the dry protein and 25 c.c. of 20% sulphuric acid are transferred to a 300 c.c. Kjeldahl flask fitted with a Hopkins condenser. The mixture is boiled gently over a micro-burner for 12 hours, after which it is cooled, diluted to 100 c.c. and thoroughly mixed. From 1-10 c.c. of the solution are transferred to a 100 c.c. volumetric flask and to it are added first 20 c.c. of saturated sodium carbonate solution and then 10 c.c. of 20% sodium sulphide solution. The mixture is well shaken and set aside while the standard cystine solutions are prepared. The standard cystine solution is made to contain 5% sulphuric acid and 1 mg. of cystine per c.c. This solution keeps indefinitely. Two standards are prepared, containing 1 and 3 c.c. respectively of cystine solution or 1 and 3 mg. of cystine. Add to each 20 c.c. of saturated sodium carbonate solution and 10 c.c. of 20% sulphide and let stand for five minutes. 3 c.c. of the uric acid reagent of Folin and Denis are then added to each standard and to the unknown digestion mixture. The three flasks are allowed to stand for 10 minutes. The contents are then diluted to 100 c.c. and the colour comparison between the unknown and the standard nearest it in colour is made in the usual manner. There is no need for any undue hurry in the making of the colour comparison for the slow fading which takes place is exactly the same in the unknown as in the case of the standard."

Folin and Marenzi describe the following procedure:—

The reagents required are:—

Uric acid reagent free from phenol and from molybdates,

A 20% solution of lithium sulphate,

A 20% solution of sodium sulphite (Merck), (to reduce the cysteine to cystine),

A 3% solution of sodium sulphite (for the dilution to the final volume),

A 20% solution of sodium carbonate,

A standard solution of cystine in normal sulphuric acid containing 1 mg. of cystine per c.c.

“Transfer from 1 to 5 grm. of accurately weighed protein to a 300 c.c. Kjeldahl flask, add 20 c.c. of 6 *N* sulphuric acid plus 2 c.c. of butyl alcohol (to prevent foaming). Connect with a small vertical condenser and boil gently on a sand bath for 18 to 20 hours. Remove the condenser and boil off the butyl alcohol. After the butyl alcohol has been removed, dilute the hydrolysate in a volumetric flask to 100 c.c. and mix. Transfer 2 grm. of kaolin to a 200 c.c. flask, add the hydrolysate, shake gently for 3 to 5 minutes and filter.

“From 1 to 5 c.c. of the nearly decolorised hydrolysate are taken for the determination.

“Transfer the required amount to a 100 c.c. volumetric flask and 2 c.c. of the standard cystine solution to another. Add 2 c.c. of freshly prepared 20% solution of sodium sulphite (Merck) to each flask and let stand for 1 minute. Then add 18 c.c. of the sodium carbonate solution to the standard and to the unknown add 18 c.c. plus 0.5 c.c. for each c.c. by which the hydrolysate taken exceeds 2 c.c. For example, if 4 c.c. of hydrolysate were taken, use 19 c.c. of carbonate solution. After the addition of the carbonate, add also 2 c.c. of 20% lithium sulphate solution and finally add, with shaking, 8 c.c. of uric acid reagent. Let stand for 3 to 5 minutes, dilute to volume (using the 3% solution of sodium sulphite) and make the colour comparison.”

Rimington (*Biochem. J.*, 1930, **24**, 1114) finds that turbidities sometimes develop on adding the reagent. These can be completely inhibited by adding 5 c.c. of 40% solution of urea immediately prior to adding the uric acid reagent. This addition of urea does not influence the final colour in any way.

Cystine and cysteine can also be determined by the iodine absorption method of Okuda (*J. Chem. Soc. Abs.*, 1924, **126**, 792; *J. Biochem. Tokyo*, 1925, **5**, 201).

**Histidine** can, under certain limited circumstances, be determined by the method of Koessler and Hanke (see Vol. VIII, p. 690).

**Tyrosine** can be determined by the method of Folin and Ciocalteu, (*J. Biol. Chem.*, 1927, **73**, 627; see also the Weiss-Millon method, Vol. VIII, p. 698). Folin and Ciocalteu's method is as follows:

"By means of a long, slender test-tube, transfer into a *new*, clean, *dry*, Kjeldahl flask (of Pyrex glass, 250 c.c.) about 1 gm. of thoroughly dried protein material. The exact weight is obtained by weighing the tube before and after the transfer. Then introduce into the flask 2 c.c. of butyl alcohol (to prevent foaming), a couple of short spirals made from silver wire or silver foil (to prevent bumping), and, finally, 4 gm. of sodium hydroxide in the form of 20% solution. Insert into the neck of the flask a condenser made from a test-tube of such a size that it fits very loosely, yet rests firmly on the flask by means of its flange.

"The mixture should be boiled for 18 to 20 hours. For this boiling, it is inadvisable to apply the flame directly to the bottom of the flasks . . . Some form of improvised air bath should be used to secure an even application of heat. An iron crucible (diameter 7 cm.) is satisfactory. The boiling will continue perfectly smoothly if the silver coils are right and provided that the condenser continues to function so that the butyl alcohol is not lost. It is not necessary to boil hard.

"At the end of the boiling period, remove the condenser, add 10 c.c. of water and continue the boiling for ten minutes, to remove the alcohol. Then remove the flame and, from a pipette, add immediately to the hot solution, drop by drop, but rather fast, 10 c.c. of 14 *N* sulphuric acid (200 c.c. of concentrated  $\text{H}_2\text{SO}_4$  diluted to 500 c.c.). It is quite essential that the first 10 c.c. of acid should be introduced into the alkaline solution while the latter is still quite hot. The addition of acid should, in fact, produce boiling. Unless the mixture becomes very hot, the silicic acid is apt to remain in colloidal solution, and the mixture will have to be discarded.

"The first 10 c.c. of acid are more than enough to neutralise the alkali in the flask. After the addition of 10 c.c. of acid shake thoroughly and cool. Then add 5 c.c. more of the 14 *N* acid to

produce the required acidity; rinse the contents into a 100 c.c. volumetric flask, dilute to volume, shake thoroughly and filter. The filtration is slow, and the funnel should be covered with a watchglass during the two hour period required to get about 60 c.c. of filtrate.

"If more than 60 c.c. of filtrate is desired, it is best to start with 2 grm. of protein material. In that case, one should add 8 grm. of sodium hydroxide and for neutralisation and acidification should use 20 c.c. and 10 c.c. of 14 *N* sulphuric acid. The acidified digest is then diluted to 200 c.c. before filtering.

"The acidified protein hydrolysates should be kept in an ice box, or at least in the dark, unless all the desired determinations can be started rather promptly, for if the hydrolysates stand around exposed to light at room temperature for many days, they soon grow dark in colour, owing to decomposition of the tryptophane.

"Transfer to a 15 c.c. centrifuge tube 8 c.c. of the protein hydrolysate and add, drop by drop, from a height of about 3 cm. 4 c.c. of a 15% solution of mercuric sulphate in 6 *N* sulphuric acid. No stirring is necessary. Let the mixture stand for two to three hours and centrifuge fairly hard for five minutes. Decant the supernatant liquid into a 100 c.c. volumetric flask, draining thoroughly and rinsing the edge of the centrifuge tube with about 2 c.c. of 0.1 *N* sulphuric acid. The amount of tyrosine remaining with the tryptophane is perhaps a shade more than could be accounted for on the basis of the amount of mother liquor in the tube. To the sediment in the tube add 10 c.c. of a solution containing 1.5% mercuric sulphate in 2 *N* sulphuric acid.

"Stir with a fine glass rod and let stand for ten minutes. Traces of precipitated tyrosine dissolve fairly easily in 2 *N* acid and the added mercuric sulphate prevents the solution of any tryptophane. At the end of ten minutes, rinse the stirring rod with 2 c.c. of the same 1.5% mercuric sulphate solution. Centrifuge again and transfer this wash liquid to the flask containing the original mother liquor, not omitting to rinse the edge of the centrifuge tube.

"The standard is prepared as follows: Introduce into a second 100 c.c. volumetric flask 5 c.c. of a standard tyrosine solution in 2 *N* sulphuric acid containing 1 mg. of tyrosine per c.c. Add 4 c.c. of the 15% mercuric sulphate solution and 12 c.c. of the 1.5% mercuric sulphate solution and about 7 c.c. of 0.1 *N* sulphuric acid.

"To the standard and the unknown must further be added 6 c.c. of 7 *N* sulphuric acid, for the total acidity in each flask should be approximately equivalent to 100 c.c. of normal acid. Heat the two flasks in boiling water for 15 minutes and then cool in cold water approximately to room temperature. Next add to each flask, with shaking, 1 c.c. of 2% sodium nitrite solution. Dilute to volume at once and make the colour comparison without undue delay, always, of course, first reading the standard against itself so as to adjust the colorimeter or the eye.

"If the standard is set at 20 mm. then 20, divided by the reading of the unknown, multiplied by 1.25 and by 5 gives the per cent. of tyrosine, provided that the hydrolysate represents exactly a 1% protein solution."

**Arginine** has recently been determined by the use of the enzyme arginase. This enzyme releases urea from the arginine, and the amount so released can be determined, either as the crystalline derivative, dioxanthylurea (Bonot and Cahn, *Bull. Soc. Biol. Chem.*, 1927, 9, 1001) or by conversion into ammonia by the enzyme urease (Hunter and Dauphinée, *Proc. Roy. Soc., B.* 1924, 97, 209, *J. Biol. Chem.*, 1925, 63, xxxix, Jansen, *Chem. Weekbl.*, 1917, 14, 214).

In Table 2 are given the percentage of arginine, lysine, histidine, cystine, tryptophane and tyrosine for a number of proteins. In most cases, the percentages for arginine, lysine and histidine have been obtained by a Van Slyke analysis and the percentages for cystine, tryptophane and tyrosine by colorimetric methods. The values for arginine, lysine, cystine and tryptophane may be regarded as the most important for forming an estimate of the food value. Compared with animal proteins, the vegetable proteins are high in arginine and low in lysine and cystine. Tryptophane and histidine are present in about the same percentage in albumins and globulins of both plant and animal origin. The prolamins, which have already been noted as low in basic nitrogen, are also deficient in tryptophane, oat gliadin and zein containing none of this important food material. Zein contains no lysine and the prolamins, as a class, are not only low in lysine, but also in histidine and, unlike the other plant proteins, in arginine.

In assessing the probable biological value of a protein from the consideration of its amino acid content, it is important to realise that proteins which are themselves of low biological value

if given as the sole protein in a diet, may have a high supplementary value when given along with others. Thus, gelatin, which alone is of little value as a nitrogen-containing food, forms a valuable supplement to many of the vegetable proteins on account of its high content of lysine.

Finally, it must also be confessed that after the chemist has made a full chemical analysis of any protein and has summed up the evidence as to its probable biological value, feeding tests remain the only final criterion. A consideration of work carried out in this direction is beyond the scope of this article and the reader is referred to the chapter by Chick in "*Recent Advances in Analytical Chemistry*" and to McCollum and Simmonds "*The Newer Knowledge of Nutrition*."

## CEREAL PROTEINS

The cereal seeds are undoubtedly the most important of the vegetable foods which contain proteins in considerable quantities. As already mentioned, the prolamins, which are of poor feeding value, form a large part of the protein of cereal seeds. The average analyses of various cereal seeds are given in Table 3 and the distribution of the protein in Table 4.

**Wheat** (*Triticum vulgare*) is the most interesting of the cereals on account of the special properties of the wheat gluten which make the flour from this cereal capable of being worked into a fermentable dough which can be permeated with gas bubbles under the action of yeast or other "leaven" and baked to make a porous, digestible loaf. Wheaten bread is the staple food in all countries where Western civilisation has made its way and its consumption is extending even now over new areas. The property of making gluten is confined to wheaten flour. The bulk of the wheat crops of the world are used for human consumption, only inferior crops going for feeding stock. Wheat grains contain four vitamins, A and E in the germ oil, B in the bran and C in the embryo.

**Barley** (*Hordeum vulgare*) is an important cereal. It is used chiefly for brewing and also largely for feeding stock. It was formerly considered that the proteins of malt were different from those of the barley grain. It has now been shown, however, that barley and malt contain the same proteins only differing in amounts, the gliadin (hordein) disappearing during the malting process and to



TABLE I

	Hausmann numbers. Nitrogen as percentage of total nitrogen				Basic nitrogen fractions as percentage of total nitrogen				Percentage in protein		Reference	
	Ammonia N	Humin N	Basic N	Non-basic N		Cysteine N	Arginine N	Histidine N	Lysine N	Nitrogen		Sulphur
				Monoamino N	Non-amino N							
<b>Cereal Proteins</b>												
<i>Wheat</i>												
Albumin (leucosin).....	6.85	2.54	20.67	69.87							16.93	1.280*
Globulin.....	7.72	1.52	37.14	53.39							18.39	
Prolamin (gliadin).....	24.61	0.58	10.97	51.95	10.70	0.86	5.45	3.39	1.33	17.66	1.027*	
<i>Barley</i>												
$\alpha$ -glutelin (glutemin).....	17.8	1.05	21.30	45.4	13.0	1.76	10.95	5.50	3.09	17.14	1.59	
$\beta$ -glutelin (glutenin).....	11.06	1.32	25.55	49.13	14.9	5.43	6.10	6.17	6.85	16.10	0.68	
Albumin.....	9.40	1.10	26.04	60.44	2.67	1.47	11.64	4.48	8.44			
<i>Oats</i>												
Prolamin (hordein).....	23.00	1.70	7.69	53.85	12.49	1.58	5.00	0.93	0.18	17.21	0.847*	
Glutelin.....	12.31	6.90	21.91	47.98	11.04					14.4		
<i>Rye</i>												
Prolamin (gliadin).....	23.78	0.79	5.40	70.27							17.66	
<i>Mate</i>												
Prolamin (zein).....	18.41	0.99	3.03	77.56							16.13	0.600*
<i>Rice</i>												
$\alpha$ -globulin.....	6.91	1.52	29.40	56.67	5.50	1.61	15.48	4.01	8.39	16.31	0.98	
$\beta$ -globulin.....	7.70	0.86	37.53	49.94	3.81	1.88	27.23	4.54	3.88	17.94	1.45	
Glutelin (oryzenin).....	10.96	1.06	30.78	54.33	2.30	1.56	20.38	3.68	5.16	16.81	0.94	
<b>Leguminous Proteins</b>												
<i>Garden pea</i>												
Globulin (legumin).....	9.3	0.83	28.3	61.5							18.04	0.385
Globulin (vicilin).....	10.0	1.23	28.8	60.0							17.05	0.200



TABLE 1.—(Continued)

	Hausmann numbers. Nitrogen as percentage of total nitrogen				Basic nitrogen fractions as percentage of total nitrogen				Percentage in protein		Reference	
	Ammonia N	Humine N	Basic N	Non-basic N		Cysteine N	Arginine N	Histidine N	Lysine N	Nitrogen		Sulphur
				Monoamino N	Non-amino N							
Spinach (spinacin).....	6.93	2.47	28.51	58.09	2.58	1.27	13.83	3.89	9.62	16.25	1.19	Chibnall, <i>J. Biol. Chem.</i> , 1924, 61, 303
<i>Caulisflower Proteins</i> (crude proteins)												McKee and Smith, <i>J. Biol. Chem.</i> , 1926, 70, 273
(a) heat coagulable.....	7.03	3.88	26.32	55.85	3.60	0.70	12.28	2.68	10.66	14.1	0.94	
(b) alkali soluble.....	6.97	3.64	31.74	48.89	8.30	1.03	13.98	6.11	10.62	13.6	0.83	

\* Osborne, *Z. anal. Chem.*, 1902, 41, 25.

[All figures from Osborne, "The Vegetable Proteins" except where reference is given.]

a less extent the glutelin (see Bishop, *J. Inst. Brewing*, 1928, **34**, 101; 1929, **35**, 316).

**Oats** (*Avena sativa*) are used in the form of oatmeal for human consumption and also as crushed or rolled oats for making porridge. The greater part of the oats crop, however, goes for feeding stock. Oats contain more fat than any other cereal and an unusually high amount of globulin for a cereal seed. The globulin, which has been called avenelin, has been obtained crystalline (Osborne and Campbell, *J. Amer. Chem. Soc.*, 1896, **18**, 542).

**Rye** (*Secale cereale*) is still grown as an important crop in Germany and other middle-European countries for making rye-bread. It was formerly grown largely in England for the same purpose, being used either alone or mixed with wheat (see "The Bread of Our Fathers" by Sir William Ashley). The tendency in modern Germany also is to mix some wheat into the rye-bread and it seems probable that the cultivation of rye is declining.

**Maize** (*Zea mais*) is an important cereal crop, particularly in tropical and semi-tropical countries. It is the staple food of the natives of Central Africa. It is largely used for human food in other countries and is important for feeding stock. The proteins of maize are not of high biological value. Half the proteins of the seed consist of the prolamins zein which is deficient in tryptophane and lysine.

**Rice** (*Oryza sativa*) is a very important cereal. It is consumed all over the world and is a staple food in China and Japan. The cultivation of rice requires a hot, moist climate and it is carried out mainly in China, Japan and some parts of India. It has recently been carried out successfully in parts of Europe. The proteins of rice are regarded as of very high feeding value. They contain from 30-40% of basic nitrogen (see Table 1). Rice is the only cereal which contains no prolamins. Apparently it contains no albumin. Nearly all the protein is of glutelin.

**Millet** (*Panicum* sp. and *Sorghum* sp.) is grown largely for human food in tropical or semitropical countries, particularly in Asia. The Indian dourra or sorghum is a variety of millet, and so is the kafir corn cultivated largely in the southern states of North America.

**Buckwheat** (*Polygonum pagopyrum*) is not a cereal crop, and its proteins differ considerably from those of the cereals. It is cultivated largely in North America and ground into a farinaceous flour. For convenience, therefore, it can be considered with the cereals.

TABLE 2

	Amino acids expressed as percentage of protein				
	Van Slyke determinations		Colorimetric determinations		
	Arginine	Histidine	Lysine	Cystine	Tryptophane Tyrosine
<i>Cereal Proteins</i>					
Wheat albumin (leucosin).....	5.94	2.83	2.75	3.20	4.76
globulin (from bran).....				1.52	2.85
prolamin (gliadin).....	3.16	0.58	0.63	1.32	1.00, 0.79*
glutenin (glutenin).....	4.2	1.0	1.92	1.56	1.72
$\alpha$ -glutelin.....	5.83	3.48	2.76	3.25	
$\beta$ -glutelin.....	3.05	3.67	5.75	7.40	
Barley, prolamin (horden I).....				1.55	1.05
prolamin (horden II).....	2.16	1.28	0.0(?)	1.47	0.44
Oats prolamin (gliadin).....				0.86	0.0
Rye, prolamin (gliadin I).....				2.04	0.36
prolamin (gliadin II).....	2.22	0.39	0.0(?)	2.61	0.75
Maise prolamin (zein).....	1.55	0.82	0.0	0.85	5.88*
glutelin.....	7.06	3.00	2.93		
<i>Millet</i> , (kafir corn) prolamin (kafirin I).....				0.55	1.17
prolamin (kafirin II).....				0.53	0.73
<i>Rice</i> , globulin- $\alpha$ .....	7.85	2.42	7.14	2.25(2.11)	2.69
globulin- $\beta$ .....	15.18	3.01	3.63	2.89(2.87)	2.32
glutelin (oryzenin).....	11.13	2.39	4.73	1.10(2.35)	2.54
<i>Buckwheat</i> , globulin.....	23.67	0.88	8.61	2.47	2.69
<i>Leguminous Proteins</i>					
Pea, albumin (legumin).....	5.45	2.27	3.03	0.83	1.76
globulin (legumin).....	11.73	1.69	4.08	0.15	0.57
globulin (vicilin).....	8.01	2.17	5.40	0.52	1.65
<i>Compea</i> , globulin (vicilin).....	7.20	3.08	4.31		
<i>Kidney bean</i> , globulin (phaseolin).....	4.87	2.62	4.58		
Total globulins.....				1.00	1.34
<i>Navy bean</i> $\alpha$ -globulin (phaseolin).....				0.58	0.94
$\beta$ -globulin (conphaseolin).....				1.53	2.70
Total globulins.....				0.59	1.52
<i>Lentil bean</i> , albumin.....				2.69	2.46
$\alpha$ -globulin.....				3.26	2.26
$\beta$ -globulin.....				0.60	0.0
Total proteins.....				2.65	1.95
					Csonka and Breeze Jones, 1927, loc. cit.
					Breeze Jones and Gersdorff, 1927, loc. cit.
					Breeze Jones and Csonka, 1927 loc. cit.
					From McCollum and Simmonds.

<i>Lentil</i> , globulin (legumin).....				0.68	0.03	
globulin (vicilin).....				0.40	0.08	
<i>Joya bean</i> albumin (legumelin).....	5.35	2.04	4.91			
globulin (glycinin).....	7.69	2.10	3.39	1.12	1.66	
<i>Nut Proteins</i>						
<i>Almond</i> globulin.....	12.16	1.87	0.72	0.85	1.37	
<i>Brasil nut</i> globulin (excelsin).....				1.84	2.59	
<i>Coconut</i> globulin.....				1.54	1.25	
<i>Hazel nut</i> (filbert) globulin.....				0.85	1.37	
<i>Walnut</i> globulin.....				2.18	2.84	
<i>Peanut</i> albumin.....				1.47	2.33	
$\alpha$ -globulin (arachin).....				1.08	0.88	
$\beta$ -globulin (con-arachin).....				3.00	2.13	
<i>Oil Seed Globulins</i>						
<i>Castor bean</i> globulin.....	13.19	2.74	1.54			
total proteins.....				1.43	2.20	
<i>Cotton seed</i> .....	13.51	3.46	2.06	1.07	2.58, 1.44*	3.64*
<i>Hemp seed</i> (edestin).....	14.17	2.10	1.65	0.97	2.48, 1.40*	4.28*
<i>Palm kernel</i> .....				1.95	0.74	
<i>Sesame seed</i> $\alpha$ -globulin.....	15.07	2.68	5.43	1.94	2.77	4.72
$\beta$ -globulin.....	15.58	3.45	3.90	1.47	2.65	4.48
<i>Green Leaf Proteins</i>						
<i>Lucerne (alfa-alfa)</i> .....	7.11	2.56	3.34	0.93†	2.86†	3.19
<i>Sprach</i> , spiracin.....	6.95	2.34	8.19	2.72†	1.85†	
<i>Cauliflower</i> proteins (a) coagulable	4.87	1.30	7.07	(2.357)	0.52	5.76
(b) alkali soluble.....	5.87	3.06	7.53		0.8	7.6

\* Polin and Marenzi, *J. Biol. Chem.*, 1930, 83, 89

† Jones, Gersdorff, Moeller, loc. cit.

(Figures for Van Slyke analyses are from Osborne, "The Vegetable Proteins" and for colorimetric determinations are from Breeze Jones, Gersdorff and Moeller, *J. Biol. Chem.*, 1934-5, 62, 188, except where reference is given.)

**Distribution of Cereal Proteins.**—The seed of cereals consists of the husk or bran, the endosperm and the embryo or germ. In wheat, the bran forms 13.5% of the seed, the endosperm 85% and the embryo 1.5%; in maize the figures are 12%, 74% and 13%, respectively. Cereal seeds contain all four different classes of plant proteins—albumins, globulins, prolamins and glutelins. Proteoses can generally be obtained from cereal seeds, but are probably derived from the proteins by the action of the enzymes of the germ. The albumins and globulins extracted from cereal seeds or ground meals are derived entirely from the embryo or germ, with the possible exception of the globulin in oatmeal, which is present in unusually large amount. The chief proteins of cereals are the *prolamins* and the *glutelins*, the reserve proteins of the seed. These proteins are confined to the endosperm and are not present in the germ (see Table 5). The endosperm of buckwheat, which, as already stated, is not a cereal, contains neither prolamins nor glutelins, the reserve proteins of the seed as well as the tissue proteins of the embryo being albumins and globulins. Table 4 shows the distribution of the different classes of proteins in cereal seeds and buckwheat. It will be noticed that rice differs from the other cereals in containing no prolamins.

The prolamins have been studied to a greater extent than the other cereal proteins, probably on account of the comparative ease with which they can be prepared pure. The gliadins of wheat and rye are very closely allied in composition and properties, but the other prolamins have very distinctive characteristics. It has already been mentioned that, as a class, the prolamins must be regarded as food proteins of low quality. Flours and ground meals, however, must not be ranked in food value on the properties of their prolamins only, but of the total proteins which they contain.

**The Gluten of Wheat.**—The glutelins are also important cereal proteins and among them glutelin of wheat is quite special in its properties. Sharp and Gortner (*Minnes. Ag. Exp. St. Tech. Bull.* 19, 1923) consider that the colloidal properties of flour depend solely on the glutelin. Glutelin and gliadin of wheat together make *gluten*, an elastic material that can be obtained as a sticky residue from wheaten flour after washing away the starch grains. It is now recognised that bread-making (as understood in modern Western civilisation) depends for its success on the properties of the gluten

TABLE 3.—AVERAGE ANALYSES OF CEREAL SEEDS (AND BUCKWHEAT)  
(Compiled from Kent-Jones and Tibbles)

	Water, %	Carbo- hydrate, %	Protein, %	Fibre, %	Fat, %	Sugars, %	Ash, %	pH
<i>Wheat, grain</i> .....	8-17	63-71	10-15	2-3	1½-2	2¼-3½	1½-2	.....
<i>Wheaten flour</i> .....	13-15	65-70	8-17					
Wheat { Endosperm.....	13.0	74.3	10.5					
{ Bran.....	12.5	43.6	16.4	0.7	0.8	2½	½	6.1-6.4
{ Embryo.....	12.5	31.2	35.7	18.0	3.5	.....	0.7	.....
<i>Barley, grain</i> .....	14.3-10.8	66.0-70.2	8.7-12.0	1.2	13.1	.....	6.0	.....
<i>Oats, grain</i> .....	9.7-15.7	53.3-62.5	8.6-12.0	2.7-5.0	1.8-2.5	up to 3.4	5.7	.....
<i>Oats, meal</i> .....	7.9-9.5	54.6-67.0	12.7-18.4	6.8-11.9	3.3-7.4	.....	.....	.....
Oats { Endosperm.....	13.4	63.5	12.3	0.8-2.2	7.6-12.3	.....	1.7-4.0	.....
{ Husk.....	6.8	52.2	2.4	1.3	7.7	.....	1.8	.....
{ Grain.....	13.4	60.23	9.5	33.4	1.3	.....	3.7	.....
<i>Rye, flour</i> .....	12.6-14.5			9.0	5.3	.....	2.6	.....
<i>Maize, grain</i> .....	12.5	66-78	3.7-8.3			1.5-2.4	0.4-1.3	6.0-6.3
{ Endosperm.....	0.0	85.0	7.0-9.5	0.9-1.8	1.5-3.5	.....	0.6-1.3	.....
{ Husk (hull).....	0.0	74.1	12.2	0.6	1.5	.....	0.7	.....
{ Embryo.....	0.0	34.7	6.6	16.4	1.6	.....	1.3	.....
<i>Rice, unhusked</i> .....	12.5	65.2	21.7	2.9	29.6	.....	11.1	.....
husked.....	11.7	77.8	6.3	7.8	2.1	.....	5.9	.....
polished.....	12.9	79.4	7.7	0.7	1.2	.....	0.9	.....
<i>Millet</i> .....	12.0	62.2	6.5	0.25	0.5	.....	0.5	.....
<i>Buckwheat</i> .....	13.0-14.5	61.2-63.6	10.2-15.2	5.8	4.6	.....	2.8	.....
				2.1-11.1	2.2-3.4	.....	2.1-2.3	.....



TABLE 4.—DISTRIBUTION OF PROTEINS IN CEREALS (AND BUCKWHEAT)

	Total, %	Albumin, %	Globulin, %	Prolamin, %	Glutelin, %
<i>Wheat</i> (Osborne & Vorhees, <i>J. Am. Chem. Soc.</i> 1894, 16, 524)	10	0.3-0.4	0.6-0.7 + 0.3% proteose	4.25	4.0-4.5
<i>Wheat embryo</i> (Osborne, & Campbell, <i>J. Amer. Chem. Soc.</i> 1910, 22, 379)		10	5 + 3% proteose	0	0
<i>Barley</i> (Osborne, <i>J. Amer. Chem. Soc.</i> 1895, 17, 539)	9-12	leucosin 0.30	1.95 (including proteose)	4.0	4.5
<i>Oats</i> (Schryver & Buston, <i>Proc. Roy. Soc. B.</i> 1926, 99, 476)			++	++	4.0
<i>Rye</i> (Osborne & Clapp, <i>Am. J. Physiol.</i> 1908, 20, 494)	17.5-12.6	0.4	1.7 (including proteose)	4.0	2.5
<i>Maise</i> (Osborne, <i>J. Am. Chem. Soc.</i> 1897, 19, 525; <i>Am. J. Physiol.</i> 1907/8, 20, 477)	10	0.45 (including proteose)		5.0	3.15
<i>Rice</i> (Rosenheim & Kajura, <i>J. Physiol.</i> 1907/8, 36, liv)	7.0	0.04	0.14	0.0	6 (about)
<i>Sorghum</i> (Kafir corn) (Johns & Brewster, <i>J. Biol. Chem.</i> 1916, 28, 59)	11.7	+	+	kafirin 7.9 (crude) 5.2 (pure)	.....
<i>Buckweat</i> (Johns & Chernoff, <i>J. Biol. Chem.</i> 1918, 34, 439) Ritthausen, <i>Der Eiweisskörper der Getreidarten, Hülsenfruchte u. Ölsamen</i> , Bonn, 1872.	6.5, 7.8 10.44	about 2	about 2	0.0	0.0

of the flour—this must be present not only in the right quantity but also in the right condition: it must be sufficiently elastic to allow the gas bubbles produced in the fermenting dough to expand, but sufficiently coherent to retain them in the dough. Much work has been done on the physical properties of gluten and it is now realised that the physical condition of this protein, like that of other proteins, depends on the  $pH$  value of the aqueous system of the dough and on the salt content. The  $pH$  value of the aqueous extracts of most flours is about 6.0–6.5 and the main salt constituents are the dihydrogen and monohydrogen potassium phosphates. It has been shown that the optimum physical condition of gluten for bread-making is at  $pH$  5.0 and it has long been known in the baking trade that the addition of acid phosphates to flour frequently improves its baking qualities. While this practice has been condemned by some people as adulteration, there seems no valid objection to the addition of carefully calculated amounts of pure phosphates with the object of controlling the physical condition of the gluten. The  $pH$  value of the dough, however, is not the only factor in determining the “strength” of the gluten; the degree of hydration of the proteins is of enormous importance. This is high in the unripe seed and is reduced during ripening. In English wheats the ripening process has not generally proceeded far enough, the gluten is still over-hydrated and the wheat, used alone, makes a “weak” flour that will not retain the gas bubbles in the dough and so makes a poorly risen loaf. On the other hand, in wheats from tropical countries, such as Indian wheats, ripening has proceeded too far and the gluten has become over-hydrated and the wheat, if used alone, makes an over-strong flour that will not stretch under the pressure of the gas bubbles in the dough and so again makes a poorly risen loaf. For a full account of “strong” and “weak” flours and on recent experiments by Kent-Jones, showing that weak flours can be strengthened by controlled heating in the dry state, the reader is referred to Kent-Jones “Modern Cereal Chemistry” and Wood “Story of a Loaf of Bread.” Success in bread-making depends on the ability of the dough to hold the gas produced by the fermenting yeast. Obviously, therefore, it depends on the suitability of the dough as a food for the yeast plant—*i. e.*, a good flour must contain sugar and sufficient diastatic enzymes to keep up a supply of sugar for fermentation and gas production.

However, diastatic activity is not generally determined for flours. For general purposes, moisture, starch, protein, fibre, fat, ash and sugar are determined. The pH values and titratable acidity of standard aqueous extracts are also of value. Flour improvers and bleaching agents are tested for separately.

### ANALYSIS OF FLOUR

The analytical procedures described below have been transcribed largely from the works of Kent-Jones, particularly "*Modern Cereal Chemistry*" and the chapter on "Cereals" in "*Recent Advances in Analytical Chemistry*, Vol. I." The author wishes to take this opportunity of acknowledging indebtedness to these two excellent text-books. The methods described for wheaten flour are equally suitable for any other flour.

**Moisture** must be determined under standard conditions. Whole wheat grains should be dried for 24 hours in a water oven. It is better, however, to crack the grains in a coffee mill, when 9 to 12 hours will give the correct moisture figure. For flours, five gm. should be taken and dried for 4-6 hours. 13.5% of moisture, as determined in the water oven, is allowed in flour, 15% if the determination be made in a vacuum oven, as is frequently done in the United States.

**Protein** is determined by the Kjeldahl method, 1 gm. is sufficient. The nitrogen figure is multiplied by a conversion factor, usually 5.7 for wheat and 6.25 for the other cereals. The factor 5.7 is only accurate for the wheat endosperm; 6.31 should be used for the bran and 5.80 for the embryo.

**Protein distribution** was formerly investigated by extracting firstly the albumins and globulins (soluble in 5% potassium sulphate solution); secondly the gliadin (soluble in 70% alcohol) and finally by calling the residual protein, glutenin. Kent-Jones recommends the determination of glutenin by a direct method.

**Albumins and globulins** are extracted by shaking 6 gm. of flour with 100 c.c. of 5% potassium sulphate solution for 1 hour. After this, stand for 30 minutes and then centrifuge or filter. Estimate the nitrogen in 50 c.c. of the filtrate by the Kjeldahl method. Nitrogen  $\times$  5.7 is taken as soluble protein.

**Glutenin** is best determined by the Blish and Sandstedt method (*Cer. Chem.*, 1925, 2, 191). 8 gm. of flour are shaken up with 50

c.c. of water and then 5 c.c. *N* sodium hydroxide is run in with shaking which is continued for half an hour. Acetone-free methyl alcohol (96-99%) is added with shaking in 50 c.c. lots until the total volume in the flask is 205 c.c. The starch settles rapidly at this stage and the clear solution can be decanted off. 50 c.c. (equivalent to 2 gm. of flour) is pipetted off and 0.2 *N* hydrochloric acid is run in to give a light olive colour with bromthymol blue as indicator. this indicates a *pH* value of about 6.4. The glutenin settles out and can be freed from the alcoholic solution of gliadin by centrifuging. The disc of glutenin can be washed in water and estimated for nitrogen.  $N \times 5.7 = \text{glutenin}$ .

Blish and Sandstedt in a recent paper (*J. Biol. Chem.*, 1929/30, 85, 195) state that this treatment with alkali alters very considerably the properties of the glutenin. They give a new method for preparing glutenin without contact with alkali.

**Gliadin** can be determined directly by extracting the residue left from the extraction with salt solution with 75% alcohol or by subtracting the figure for soluble proteins plus glutenin from the total protein.

**Gluten** determinations for different flours are only comparable if always carried out by the same worker. The determination of gluten is rather out of favour. Kent-Jones, however, considers the determination useful. 20 gm. of flour should be made into dough and placed on a piece of fine silk in a bowl of tap water and allowed to stand for 1 hour. The tap should then be turned on at a flow of 250 c.c. per minute and the dough kneaded for 10 minutes. The silk serves to catch the particles that break off. The gluten should be dried on a weighed filter paper for 24 hours in a water oven.

**Starch** is determined by mixing 5 gm. of flour with 100 c.c. of water and gelatinising on a water bath. The mixture is cooled to 55° C. and 1 gm. of taka-diastrase is added. After 1½ hours, the solution is cooled, made up to 250 c.c. and filtered. 200 c.c. of the filtrate are boiled with 20 c.c. of dilute hydrochloric acid under a reflux condenser for 2½ hours, cooled, made nearly neutral with 10% sodium hydroxide solution and finally neutralised to methyl orange with 10% sodium carbonate. The volume is made up to 500 c.c. and 25 c.c. are taken for a Fehling estimation. Corrections must be made for the taka-diastrase and the sugar in the flour.

**Sugar** is determined by taking 30 grm. of flour and 160 c.c. of water, warming to  $27^{\circ}$  and keeping at that temperature for 1 hour. Diastatic action is inhibited by the addition of 6 c.c. of 15% sodium tungstate solution and 20 drops of concentrated sulphuric acid. The volume is made up to 200 c.c. and the solution filtered. A direct Fehling determination gives maltose; 50 c.c. of the filtrate boiled for 10 minutes with 1 c.c. of concentrated hydrochloric acid and neutralising with sodium carbonate can be used for determination of maltose plus cane sugar.

**Cellulose** or **fibre** is determined by boiling 5 grm. of flour with 150 c.c. of water for 5 minutes, adding 50 c.c. of 12% sulphuric acid, hydrolysing on the water bath for 30 minutes and then boiling for 30 minutes. The solution is filtered and the residue drained and washed with 60 c.c. of 4% sodium hydroxide solution, which is poured twice through the filter. The residue is then washed with a further 30 c.c. of the boiling alkali followed by 500 c.c. of boiling water. The cellulose is transferred to a platinum dish, dried at  $100^{\circ}$  C. and weighed.

**Fat** is determined in flour by extraction with ether in a Soxhlet apparatus. The flour should previously have been dried for 1 hour in a water oven.

**Ash** gives total bases and phosphates. High grade flour has an ash content of 0.5%, low grade up to 1%. Kent-Jones gives the following analysis for the ash of wheaten flour:  $K_2O$ , 37.04%;  $MgO$ , 6.12;  $CaO$ , 5.53;  $Fe_2O_3 + Al_2O_3$ , 0.36;  $P_2O_5$ , 49.11; and  $SO_3$ , 0.40%. The mineral matter in flour is present mainly as  $KH_2PO_4$ .

**Calcium** is determined by dissolving the ash in dilute hydrochloric acid and converting into the oxalate. It can be determined directly on saccharified flour (see next paragraph). Calculated as calcium oxide, 0.015 to 0.02% should be found in flour.

**Sulphate** must be determined by boiling 20 grm. of flour with 250 c.c. of water and 15 c.c. of hydrochloric acid (sp. gr. 1.16) for 1 hour. The filtrate is known as saccharified flour, and sulphates are determined by precipitation with barium. 0.011 to 0.061% of sulphate should be present in flour.

**Soluble phosphates** are determined by extracting 20 grm. of flour with 200 c.c. of water and adding ammonium molybdate to 50 c.c. of the filtered extract.

**Acidity** is determined by mixing 5 gramm. of flour with carbon dioxide-free water and titrating with  $N/20$  potassium hydroxide, using phenolphthalein as the indicator. The results are usually expressed as potassium dihydrogen phosphate. 1 c.c.  $N/20$  potassium hydroxide is equivalent to 0.0068 gramm. of potassium dihydrogen phosphate.

**Alkalinity** is determined similarly, using  $N/20$  sulphuric acid and methyl orange as indicator. 1 c.c.  $N/20$  sulphuric acid is equivalent to 0.00435 gramm. of di-potassium hydrogen phosphate.

**pH value of extracts** can be taken by shaking 10 gramm. of flour with 100 c.c. of carbon dioxide-free water for 45 minutes and filtering. The clear extract can be determined electrometrically or colorimetrically. Methyl red (pH 4.2–6.3) and bromo-cresol purple (pH 5.2–6.8) are useful for this purpose. Straight run flours usually give a pH of 6.10–6.25, patent flours 5.9–6.0.

**Mineral improvers** can be found by shaking the flour with chloroform or carbon tetrachloride. The flour floats and the minerals sink. They can be separated and analysed for persulphate, bromate or phosphate.

For further methods of examining flour, in particular for the addition of phosphates or other added improvers, for the presence of arsenic, for gas production and colour, the works of Kent-Jones should be consulted.

### Proteins of Leguminous Seeds (Pulses)

The dried, ripe seeds of leguminous plants, known collectively as pulses, are very valuable as food material on account of their high proportion of protein. They also contain a large percentage of carbohydrate. The common pulses belong to a number of botanical genera, classed together roughly as peas, beans and lentils. Peas and beans are also eaten in the unripe condition as green vegetables.

The percentage of proteins in pulses is higher than in cereals. The proteins are present partly as the tissue proteins of the embryo and partly as reserve proteins. Only *albumins* and *globulins* are present, the globulins, in particular, being present in large amount and apparently forming the whole of the reserve protein. Frequently, two different globulins can be isolated from the same plant species; the common garden pea (*Pisum sativum*), for instance,

contains an albumin, legumelin, and two globulins, legumin and vicilin; the kidney bean (*Phaseolus vulgaris*) contains an albumin, phaselin, and two globulins, phaseolin, which has been obtained crystalline (Osborne, *J. Am. Chem. Soc.* 1894, 16, 633, 703, 757; Finks and Johns, *J. Biol. Chem.*, 1920, 41, 375) and conphaseolin. The globulins of pulses contain a fairly high proportion of basic nitrogen, but in spite of this, some of them have a poor feeding value.

The garden pea (*Pisum sativum*) is grown all over the world. Most of the cultivated peas are sub-varieties of a single species.

The broad bean (*Vicia Faba*), with its closely allied subvarieties, the field bean and the horse bean, is also cultivated all over the world, largely as food for cattle. Bean flour from field beans has been used on many occasions in times of dearth, mixed with wheaten flour, for bread-making. The proteins of beans of the genus *Vicia* are very similar to those of peas. They are referred to under the names of legumelin, legumin and vicilin.

The cow pea (*Vigna catjang*) is really a bean. It is grown largely for stock feeding. Its proteins are similar to those of the genus *Vicia*. Vignin is the chief globulin of cow peas.

The kidney bean (*Phaseolus vulgaris*) and allied sub-varieties the haricot or French bean, is also cultivated widely and used all over the world for human food. The lima or sugar bean (*Ph. lunatus*) is a tropical plant. The dried beans are eaten largely in the United States. The Mung bean (*Phaseolus* sp.) is also of Oriental origin and has become popular in the United States. All the beans of the genus *Phaseolus* have a similar composition and their proteins are closely allied. The globulins of *Phaseolus* beans are called phaseolin and conphaseolin.

The velvet bean (*Stizolobium*) is another tropical plant grown in the Southern States (Georgia velvet bean, *S. deeringranium*) and in China (*S. niveum*). The chief globulin of *Stizolobium* beans is called stizolobin.

The lentil (*Lens esculenta* or *Ervum lens*) has been cultivated round the Mediterranean and in the East since prehistoric times. Lentils are eaten all over the world.

The soya bean (*Glycine hispida*) is grown in China and Japan and forms a staple food of these countries. The soya bean has an unusually low percentage of carbohydrate compared with other pulses and a high percentage of protein and fat. For this reason,

soya beans are sometimes used for making diabetic bread. The proteins of the soya bean are of comparatively high feeding value.

The *butter bean* (*Vateria indica*) is not a true legume but is the seed of a tree belonging to the natural order, *Dipterocarpaceae*. It is grown largely in India, but the dry beans are eaten all over the world.

Table 7 gives the analysis of the commoner pulses. The analysis of pulses presents no special problems, the proteins being extracted from the ground material by a 10% solution of sodium chloride.

TABLE 5.—ANALYSIS OF PULSES

(Taken from Tibbles)

	Water, %	Carbo- hydrate, %	Protein, %	Fibre, %	Fat, %	Ash, %
Dried peas ( <i>Pisum sativum</i> ) . . .	6.9-15.0	58.0-67.4	20.4-28.0	1.2-7.9	0.8-1.3	2.2-4.3
Dried beans (broad bean, horse bean, field bean) ( <i>Vicia faba</i> )	8.40	58.85	29.05	1.05	2.0	3.65
Cow peas ( <i>Vigna catjang</i> ) . . . . .	13.00	60.80	21.40	4.10	1.40	3.40
Dried beans (kidney, haricot or French bean) ( <i>Phaseolus vulgaris</i> ) . . . . .	0.6-15.5	57.2-63.5	19.9-26.6	3.2-7.2	1.4-3.1	2.7-4.4
Mung bean ( <i>Phaseolus</i> sp.) . . . . .	8.83	48.54	22.64	4.53	1.34	2.85
Lima beans ( <i>Phaseolus lunatus</i> ) . .	10.4	65.9	18.1	...	1.5	4.1
Butter bean ( <i>Vateria indica</i> ) . .	10.5	62.6	20.6	...	2.0	4.2
Lentil ( <i>Lens esculenta</i> or <i>Ervum lent</i> ) . . . . .	10.0-20.4	56.0-62.4	20.3-24.2	2.9-3.5	0.5-1.4	1.9-2.6
Soya beans (black) ( <i>Glycine hispida</i> ) . . . . .	8.25	14.18	36.35	5.43	17.2	4.75

## Nuts

Nuts contain a very large proportion of protein and fat. Most of them contain little carbohydrate, though the sweet chestnut forms an exception, having a high percentage of the latter and a correspondingly low percentage of the two first-mentioned constituents. The almond contains no starch. Nuts contain a very high proportion of fibre and are, therefore, difficult of digestion. Some of them, for instance, the almond, coconut and hazel nut, are pressed for their oil. An analysis of some of the common nuts is given in Table 8.



The proteins of nuts have not been studied in great detail. Those that have been isolated have the characteristic properties of *globulins*. Excelsin, the globulin of the Brazil nut has been obtained crystalline (Osborne, *Amer. Chem. J.* 1892, 14, 662).

*Peanuts* or monkey nuts (*Arachis hypogæa*) are not nuts according to the ordinary meaning of the term but are the seed pods of a leguminous plant which is peculiar for its habit of pushing the pods into the ground to ripen, hence the alternative name, earth-nuts.

TABLE 6.—ANALYSIS OF NUTS (KERNELS ONLY)  
(Taken from Tibbles)

	Water, %	Carbo- hydrate, %	Protein, %	Fibre, %	Fat, %	Ash, %
Sweet almonds (fresh).....	27.7	10.0	16.5	41.0	2.8	2.0
(dried).....	2.0-5.3	12.8-21.4	16.6-25.3	1.6-2.5	48.9-60.0	1.6-2.5
Brazil nuts (shelled).....	5.28	3.78	18.0	4.22	66.1	2.65
Chestnuts (fresh shelled).....	29.2-53.8	36.9-54.0	4.1-8.1	1.4-2.5	2.0-10.8	0.7-1.8
(dried).....	4.8-6.6	65.7-80.3	8.2-13.5	2.4-3.0	3.9-15.3	1.5-2.9
Coconuts (fresh).....	19.2	9.7	5.35	13.8	51.0	1.14
(dried).....	2.8-4.3	24.1-39.0	6.0-6.5	.....	51.0-63.7	1.2-1.4
Hazel nuts (fresh).....	48.0	11.5	8.0	2.5	28.5	1.5
(dry).....	3.7	9.7	14.9	3.2	66.4	1.8
Hickory nuts.....	3.7	11.4	15.4	.....	67.4	2.1
Peccans (dry).....	3.0	13.3	11.0	.....	71.2	1.5
Walnuts (fresh).....	44.5	9.4	12.0	0.8	31.6	1.7
(dry).....	4.7	7.4	15.6	7.8	62.6	2.0
Peanuts ( <i>Arachis</i> nuts) . . .	4.9-13.2	15.3-40.4	19.5-29.1	2.0-3.0	32.3-48.8	1.9-2.4

### Oil Seeds

The seeds of many plants contain oil in sufficient quantities to make its extraction commercially profitable. These seeds are rich in reserve proteins and the pressed seed cakes from which most of the oil has been removed are of great value as cattle foods. Certain oil seeds, however, contain proteins which are poisonous. The best known of these is *ricin* from the castor oil bean. In estimating the value of pressed seed cake of unknown composition, it may be sometimes necessary to test for the presence of *ricin*.

**Ricin** is a soluble albumin. The presence of the toxic *ricin* in cattle food can be tested by a serological technique (Mooser, Landw.

Vers. Stat., 75, 107; *Chem. Cent.*, 1911 (2), 638). 10 grm. of the material are extracted for 24 hours at 37° with 100 c.c. of glycerol and to the filtered extract are added 10 times its volume of an equal mixture of alcohol and ether. The resulting precipitate is filtered off, washed with alcohol and ether, dried at 37° or extracted with 200 c.c. of 10% sodium chloride solution. The clear filtrate from this extract is used for a precipitin reaction with a specially prepared ricin-immune serum.

**Protein Content of Oil Seeds.**—The proteins of oil seeds, like the proteins of nuts, are mainly globulins. Edestin, the globulin of hemp seed, has been obtained crystalline.

Globulins have been extracted from the seeds of numerous plants, but for details of these proteins and references to the original literature the reader is referred to Osborne.

TABLE 7.—THE PROTEIN CONTENT OF VARIOUS OIL SEEDS  
(From Smetham, *Analyst*, 1914, 39, 481)

	Albuminoids (= N × 6.25) as percentage of weight
Cacao (Guayaquil).....	15.38
Cacao shells.....	18.81
Calabash seeds.....	31.37
Canary seed cake.....	17.35
Candle nuts.....	22.13
Candle nut cake.....	42.87
Castor oil seeds.....	17.75
Coconut.....	5.44
Coconut cake (English).....	21.19
Cottonseed (Egyptian).....	19.56
Cotton cake (undecorticated) (Egyptian).....	23.01
Dhomba seed.....	6.88
Dhomba meal (extracted).....	20.75
Arachis nut husks (hand picked).....	6.01
Arachis nut and Kurdee cake.....	38.62
Hemp seed cake.....	32.38
Hemp seed meal.....	34.75
Linseed (Turkish).....	20.94
Linseed cake (English, average).....	20.50
Linseed cake (Japanese).....	35.88
Linseed meal (Japanese).....	33.12
Niger seed cake.....	34.06
Palm kernels.....	9.63
Palm kernel cake.....	15.89
Palm kernel meal.....	16.18
Poppy seed cake.....	35.38
Poppy seed meal.....	39.50
Rape seed.....	22.00
Rape seed meal (extracted).....	35.42
Sesame seed cake (Egyptian).....	44.75

An important oil-containing seed is the *cocoa-bean*, the seed of *Theobroma cacao*. This plant grows only in the tropics. The prepared seeds of the cocoa tree are used in enormous numbers for making cocoa and chocolate. Tibbles gives the following average analysis of cocoa-beans (taken from analyses by Ridenour): Fat, 42.9; theobromine, 0.97; protein, 10.5; glucose and saccharose, 2.6; starch, 4.2; lignin, 5.9; cellulose, 14.4; extractives, 9.3; moisture, 5.1; ash, 3.7%. In preparing commercial cocoa, a large part of the fat or cocoa butter is removed by hydraulic pressure and the ground material is sometimes mixed with sugar or starch. The high nitrogen content of commercial cocoas makes them useful as foods. Tibbles gives the protein content of commercial cocoas ( $N \times 6.25$ ) as from 4.3–13.3%. Nothing is known of the nature of the proteins, but the food value has been stated to be low.

The protein contents of a number of oil seeds and oil cakes are given in Table 7.

### Roots and Tubers; Fruits; Fresh Vegetables

With the exception of the unripe seeds of peas and beans, which are eaten as green vegetables, these parts of plants contain only a low percentage of protein. A globulin, tuberin, has been isolated from the tubers of potatoes. It is probable that all roots and tubers which are modified to form food reservoirs contain both albumins and globulins as reserve proteins. Nothing is known of the proteins of fruits. The proteins of green vegetables and of the green leaves of plants have only been studied in recent years. Proteins from the leaves of lucerne (alfa-alfa), spinach, maize (green corn) and cabbage have recently been analysed by Chibnall; Chibnall and Nolan, and Osborne (see Tables 1 and 2). These proteins are coagulated irreversibly by heat and strong alcohol. They are insoluble in water, with minimum solubility between pH 4.0 and 4.6, soluble in dilute acid or alkali, but are precipitated from their solutions by traces of salts. They are probably closely allied to the globulins. Two proteins have also been isolated by McKee and Smith from cauliflowers (see Tables 1 and 2).

The feeding value of the proteins in fruits and green vegetables for humans on ordinary mixed diet is probably not of any particular importance in the diet, and the same is probably true for stall-fed animals which are receiving a ration of oil-cake or cereal seeds.

For animals at pasture, the constitution of the proteins in grass and fodder crops, becomes of considerable significance and further work on these proteins is greatly to be desired.

A number of analyses of fruits and vegetables for so-called "albuminoid" content is given in Table 8. The figures for "albuminoids" are taken as equal to nitrogen multiplied by a factor. In green leaves and other metabolically active parts of plants, the true value for protein may however be considerably less.

TABLE 8.—PROTEIN CONTENT OF ROOTS, FRUITS AND FRESH VEGETABLES

(From König, average values)

	Albuminoids (= N × 6.25) as percentage of weight
<i>Roots.</i>	
Potatoes.....	1.99
Jerusalem artichokes.....	1.89
Yam (sweet potato).....	1.57
Mangolds (beetroot, garden beet).....	1.26
Beetroot (sugar beet).....	1.24
Carrots.....	1.18
Swedes.....	1.39
Turnips.....	1.12
<i>Fruits.</i>	
Apple.....	0.30
Pear.....	0.35
Plum.....	1.01
Cherry.....	1.29
Gooseberry.....	0.47
Strawberry.....	0.59
Grape.....	1.01
<i>Fresh Vegetables.</i>	
Melon.....	0.84
Pumpkin.....	1.10
Tomato.....	0.95
Fresh garden peas.....	6.59
Fresh broad beans ( <i>Vicia</i> ), seeds only.....	5.43
Runner beans ( <i>Phaseolus</i> ) including the green pod.....	2.72
Asparagus.....	1.95
Cauliflower.....	2.48
Savoy cabbage.....	3.31
Spinach.....	3.71
Lettuce.....	1.41
Fresh mushrooms.....	4.88
<i>Preserved Vegetables.</i>	
Bottled peas.....	3.61
Bottled beans (haricots verts).....	1.11
Tinned tomatoes.....	1.29

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# THE PROTEINS OF MILK

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The number of proteins which have been described as occurring naturally in milk has varied between one and ten or more. This wide discrepancy can be accounted for in several ways, the more important being the great difficulty in separating these bodies, the difficulty in preparing them in a pure state, and the possibility, which amounts in several cases to certainty, that the naturally occurring proteins are broken down or modified either by decomposition in the milk itself or by the action of the reagents by which they are separated. It is only within comparatively recent years that information has been obtained which has the slightest claim to reliability, but it can be said with some certainty that, although even yet complete knowledge has not been obtained, particularly in milk other than cow's milk, the main proteins have been characterised definitely and that we have a fairly complete qualitative knowledge of the greater part of the nitrogenous constituents of milk, although the quantitative aspect is still in an unsatisfactory condition for reasons which will be dealt with below.

Of the large number of proteins which are alleged to have been isolated from milk, only three can be said to have definite individual existence in the natural article. One or two others may exist in more or less minute amounts but this is uncertain.

## DETERMINATION OF TOTAL PROTEINS

**1. The Kjeldahl Method.**—The total nitrogen is determined in the usual manner by the Kjeldahl method as follows:

10 grm. of milk, 25 c.c. of pure sulphuric acid, a small crystal of copper sulphate, and 10 grm. of potassium sulphate are placed in a Kjeldahl flask, and the whole boiled until colourless. The solution is then cooled, diluted, and the ammonia determined by distillation in the usual manner. The number of c.c. of  $N/10$  acid used,

multiplied by 0.014, gives the percentage of nitrogen; or multiplied by 0.08932, gives the percentage of proteins.

As both casein and albumin—the two proteins which form by far the greater portion of the nitrogen compounds of milk—contain about 15.67% of nitrogen, the total proteins may be found by multiplying the percentage of nitrogen by 6.38. This factor has been generally accepted for many years but many workers (*e. g.* H. D. Richmond, *Analyst*, 1908, **33**, 179) consider that it is slightly low, and that 6.39 or 6.40 gives results nearer to the truth. Probably 6.39 is the best figure to take, but the more usual one is 6.38 which is that adopted by the A. O. A. C.<sup>1</sup>

A slight error, which tends to give results on the high side, is introduced by this process, since the non-protein nitrogen is returned as proteins. In general, however, the difference due to this factor is quite small, and in any case the process gives comparative results, as practically the whole of the published figures for total proteins in milk have been obtained by this process.

**2. The Ritthausen Method.**—Ten grm. of milk are diluted to about 100 c.c. and 5 c.c. of Soxhlet's copper sulphate solution containing 69.278 grams of crystallised copper sulphate per litre added; a solution of caustic soda (25 grm. per litre) is added, drop by drop, till the solution is nearly neutral; the precipitate settles rapidly. An excess of alkali must be avoided, as it prevents precipitation of the proteins. The precipitate is allowed to settle, and the supernatant liquid poured off through a tared filter or Gooch crucible. The precipitate is washed several times by decantation, and finally on the filter. The filter or crucible is washed once with strong alcohol, and then several times with ether, preferably in a Soxhlet extractor; it is then washed with strong alcohol from a small wash bottle, using the jet to distribute the precipitate over the filter. The filter or crucible and its contents are dried in an air oven at a temperature of 130° C and weighed; the filter or crucible and precipitate are incinerated in a porcelain capsule. The weight of the residue, minus that of the ash of the filter, is subtracted from the weight of the dried precipitate, the difference being the proteins.

In place of washing the precipitate with alcohol and ether, the whole may be weighed as proteins and fat, and the fat (separately determined) subtracted. The method is not so accurate as that of

<sup>1</sup> Later investigators have suggested a lower figure, 6.34.

Kjeldahl for several reasons. In the first place copper sulphate does not precipitate quite the whole of the proteins, whilst other errors are introduced by the oxidation of the organic phosphorus of the casein on ignition to phosphoric acid and the difficulty of dehydrating the copper hydroxide which is precipitated along with the casein. For this reason other precipitants have been suggested by F. Bordas and Toutplain (acetone, *Compt. rend.*, 1906, **142**, 1345). Trillat and Sauton (acetic acid and formalin, *Ann. Chim. analyt.*, 1906, **11**, 205) R. Malenfont (Alcohol and acetic acid, *J. Pharm. Chim.*, 1912, **6**, 390) W. H. Welker and H. L. Marsh ("Alumina Cream," *J. Amer. Chem. Soc.*, 1913, **35**, 823) and by B. Pfyl and R. Turnau (Carbon tetrachloride, *Analyst*, 1914, **39**, 399).

L. J. Harris (*Proc. Roy. Soc.*, 1925, **97**, 364) determines proteins volumetrically by measuring the amount of acid or alkali necessary to titrate a given volume of milk from one  $pH$  to another. 10 c.c. of milk are titrated with  $N/10$  HCl from  $pH$  6.7 to  $pH$  4, and a curve drawn. The number of c.c. required between  $pH$  6.65 and  $pH$  5.2 is numerically equal to the percentage of proteins in the milk. This method is an extension of the acidimetric methods given under casein on page 53.

The idea underlying these precipitation methods is the desire to rely on an absolute method rather than the indirect one of nitrogen determination. It is to be doubted, however, whether the precipitation methods yield results of any greater accuracy in the present state of our knowledge.

**3. Direct Volumetric Methods.**—Such methods depend upon the power exhibited by proteins of combining with certain reagents. The best known and probably the one most generally applicable is that known as the "Aldehyde figure." This was originally due to R. Steinegger (*Z. Unters. Nahr. Genussm.*, 1905, **10**, 659), and is now usually carried out by the modification suggested by Richmond. This is as follows: 10 c.c. of milk are neutralised with  $N/11$  strontia solution to phenolphthalein, 2 c.c. of 40% formaldehyde solution are then added, and the mixture again titrated until the same shade of pink is reached as in the first titration. The acidity developed by the addition of the formaldehyde, less that due to the formaldehyde itself (separately determined), calculated as degrees, gives the aldehyde figure. The aldehyde figure, expressed in degrees of acidity obtained with strontia solution, (*i.e.*, the number of c.c. of



N alkali to neutralise 1,000 c.c. of milk), multiplied by 0.710, gives the percentage of proteins. The figure must be taken as approximate only, as casein and albumin require different factors.

W. C. de Graff and A. Schaap (*Ann. Falsif.*, 1903, 6, 149) give reasons for the use of soda in place of strontia and deduce a somewhat different factor.

### THE AMOUNT OF TOTAL PROTEINS IN MILK

Whilst, as will be shown later, there is still considerable uncertainty as to the amount of individual proteins present in milk, there is a mass of more or less reliable information concerning the *total* protein content. The average amount of total proteins ( $N \times 6.38$ ) present in milk has been given by different observers as varying from 3.2 to 3.85, but this variation is doubtless explained to a certain extent by the fact that the figures refer to the milk of cows in different countries under widely varying conditions. It may be taken that the average figure for cattle in England is about 3.4 (Richmond and others) and for cattle in America about 3.2 (Van Slyke).

The actual amount of total proteins present in any sample of milk will depend upon several factors, any one of which is itself probably complex and made up of several secondary factors, some of which are either unknown or whose effect is little understood. The primary factors may be taken as:

- a. Number of cows from which the sample is obtained.
- b. Breed of cows.
- c. Environment of cows.
- d. Period of lactation and age of cows.

These different factors may be discussed shortly under different headings:

(a) **Variation Due to Number of Cows.**—The amount of total protein found in the milk of individual cows has varied somewhat widely. Thus J. F. Tocher ("Variations in the Composition of Milk" Edinburgh, 1925) found 1.66% to 4.08%, H. D. Richmond ("Dairy Chemistry," 3rd Edition, London, 1920) gives 3.11% to 6.28%, whilst L. L. Van Slyke (*J. Amer. Chem. Soc.*, 1908, 30, 1166) found 2.19% to 8.56%. Some of these figures must be regarded as abnormal and the usual variations taken as from 2.6% to 3.8%. The variations found for herds will depend, of course, to a great

extent on the size of the herd, the larger the herd the less the variation from the average, unless some special selection factor is operating in the case of a particular herd. It may be accepted that the variation usually found in a herd of ten or more cows is from 3.1% to 3.5% and that values outside these are the exception rather than the rule.

(b) **Variation Due to Breed of Cows.**—L. L. Van Slyke (*loc. cit.*) has found the proteins to vary, on the average, from 2.2% in the case of Holstein-Friesian cows to 3.9% in the case of Devon cows. J. F. Tocher found that Ayrshire cows contained 3.3% of proteins, Friesian cows 3.1%, and other cows 3.2%. The following figures have been obtained at the New Jersey Agricultural Experimental Station:

Breed	Proteins, per cent
Ayrshire . . . . .	3.5
Guernsey . . . . .	3.9
Holstein . . . . .	3.3
Jersey . . . . .	4.0
Shorthorn . . . . .	3.3

(c) **Environment of the Cow.**—From the published figures it is not always easy to separate variations due to environment and those due to breed, as figures for each particular breed kept in different countries and under different conditions in the same country are not numerous. The following average figures given by different observers working in different countries are at least suggestive that the environment of the cows has some effect on the amount of proteins contained in the milk. The word "environment" is here used to include all those factors, such as climate, food, housing, treatment, etc., which may influence in any way the quality of the milk.

Observer	Proteins, per cent
Hogan and Azadian . . . . .	3.6
L. L. Van Slyke . . . . .	3.2
Babcock . . . . .	3.8
Richmond . . . . .	3.4
Oliver . . . . .	3.9
Fleischmann . . . . .	3.5
Willoughby . . . . .	3.7

(d) **Period of Lactation and Age of Cow.**—L. L. Van Slyke found that, in general, the amount of proteins in milk increased as lactation advanced, the amount of increase as between the former and latter

weeks being of the order of 0.6%. This is supported by J. F. Tocher (*Analyst*, 1926, 51, 606). It may be observed that the change, in the case of human milk, is in the opposite direction.

### THE VARIATION OF PROTEINS WITH OTHER CONSTITUENTS

From the analysis of a large number of milks Vieth has deduced that the ratio between lactose, protein and ash is as 13:9:2. This ratio gives results which are very near the truth in the case of the mixed milk of herds having solids-not-fat between about 8.4 and 9.2. It does not hold for unwatered milks having percentages of solids-not-fat outside this range, nor for the milks of individual cows. It has frequently been stated that a deficiency of solids-not-fat is usually due to deficiency of lactose, and that a high figure for solids-not-fat is due to an increased quantity of proteins. This may be correct in the case of herds, but it is not in the case of individual cows.

H. D. Richmond (*loc. cit.*, p. 299) finds that the ash may be deduced from the formula:  $\text{Ash} = 0.36 + 0.11 \text{ Proteins}$ . H. C. Sherman (*J. Amer. Chem. Soc.*, 1903, 25, 132) modifies this formula to:  $\text{Ash} = 0.38 + 0.10 \text{ Proteins}$ .

Timpe has suggested that the percentage of proteins can be calculated from the fat by the formula:  $\text{Proteins} = 2 + 0.35 \text{ Fat}$ . Richmond's (*loc. cit.*, p. 291) results show this formula to be quite inaccurate, although Richmond and other observers agree that the proteins tend to be higher when the fat is higher. J. F. Tocher (*Analyst*, 1926, 51, 606) also agrees with this and finds that the correlation is largely due to the casein.

### THE COMPOSITION OF COLOSTRUM

Colostrum, or the secretion of the udder for a few days before and after calving, is quite different in quantitative composition from milk. The total proteins may be as high as 25% and this figure falls rapidly after calving, the normal milk being secreted in the course of a few days. The composition of the proteins is not the same as for normal milk, their great increase being due to much higher proportions of globulin (*vide infra*) than usual.

## THE COMPOSITION OF MILKS OTHER THAN COWS' MILK

The milks of different mammals may be roughly divided into two classes—those which are curdled by rennin and those which are not. This difference may be due to variation in the amount or the constitution of the proteins present and to the composition and content of the mineral salts. The former class includes the milk of cows, goats, ewes, and buffaloes, whilst in the latter class are those of women and mares. The probability that this difference in behaviour is due, not so much to differences in the proteins but to difference in the inorganic salts present, has been considerably supported by F. Demuth (*Biochem. Z.*, 1924, 105, 144) who has shown that coagulation of human milk can be brought about constantly and rapidly in the presence of suitable buffer mixtures. He finds that the optimum pH is about 4.5. H. W. Dudley and H. E. Woodman (*Biochem. J.*, 1915, 9, 97) have found that the proteins of cows' milk and ewes' milk have their amino acids differently arranged.

The following table gives the variations usually found for total proteins in the milk of various mammals.

Source	Percentage of proteins		
	Average	Usual limits	Extremes recorded
Woman.....	1.4	1.0-2.5	0.5-5.5
Buffalo.....	4.2	4.0-5.0	3.8-5.4
Ewe.....	6.0	4.4-6.6	4.4-8.8
Goat.....	4.3	.....	3.1-6.6
Mare.....	1.8	1.6-2.5	1.5-3.0
Ass.....	1.7	.....	1.3-1.9
Mule.....	1.6	.....	.....
Sow.....	7.0	.....	.....

Human milk differs from cows' milk in that as lactation proceeds the percentage of total proteins falls from about 2.1 to 1.3. This has been confirmed by several observers (vide J. A. Gardner and F. W. Fox, *The Practitioner*, 1925, 114, 153, and G. D. Elsdon, *Analyst*, 1928, 53, 78).

## THE NATURE OF THE PROTEINS OF COWS' MILK

It has already been mentioned in the introduction to this section that there has been much uncertainty as to the nature and amount of proteins present in cows' milk. Duclaux has stated that there is only one protein in milk, casein or caseinogen (*vide infra*), whilst other observers have discovered (probably many of them erroneously) from two to ten. It is probable that the proteins actually present in fresh cows' milk amount to three or four, or at most five. The three that are almost certainly present are casein, albumin and globulin. The other two which may be present are the mucoprotein of Storch, which is thought by him to surround the fat globules (R. W. Titus, H. H. Sommer and E. B. Hart, *J. Biol. Chem.*, 1928, **76**, 237 consider that this is closely related to, if not identical with, casein, the most marked distinctions between the two being the lower solubility and the darkening of its solution with sodium hydroxide of the former; they suggest that this portion is really casein contaminated with some unknown substance) and the alcohol-soluble protein of Osborne and Wakeman (*J. Biol. Chem.*, 1918, **33**, 7).<sup>1</sup> This latter has not yet been studied completely and in any case is only present to a small extent.

The greater part of the proteins of milk consists of "Casein." (For the nomenclature of this body see under "casein" below.) Thus H. M. Hoyberg (*Z. Fleisch. Milchhyg.*, 1925, **35**, 381) states that 76% of the total nitrogen of milk exists as casein, as compared with Fleischmann's figure of 85% for German milk. This figure has been variously given by different observers as varying from 75% to 90%, the variations being due partly to natural fluctuations in composition and partly to differences in the methods of separation.

Of the remaining proteins, albumin—frequently known as lactalbumin to distinguish it from similar compounds obtained from other animal products—forms the greater part, whilst a smaller quantity of globulin (lactoglobulin) is also present. Globulin is present in much larger quantities in colostrum.

These three proteins are distinguished by their reactions with reagents. Thus "casein" is precipitated from milk by means of acetic acid, whilst albumin and globulin are not. Globulin is precipitated by saturation of its solution with magnesium sulphate, whilst

<sup>1</sup> Cf. Grimmer *Milch Forsch.*, 1926, **3**, 495. G. M. Moir suggests that this body is really casein without its phosphorus.

albumin is not. By such methods are the proteins separated and identified.

## CASEIN

**Nomenclature.**—The term “casein” was at one time given indiscriminately to the curds produced in milk by various methods. It is now known that these products differ in composition according to the method of production, and that they differ from the protein as present naturally in milk. Thus the curd produced by renna<sup>s</sup>e (or rennin, rennet or chymase, an enzyme secreted by mammals and usually obtained from the fourth stomach of the calf) is quite different in its salt content from that produced by acid.

Halliburton has given the name *caseinogen* to the protein in milk, restricting *casein* to that produced by rennase. L. L. Van Slyke<sup>1</sup> states that “*Casein* or *free casein* is the base free or uncombined protein, *calcium casein* or *calcium caseinate* is the neutral compound that is believed to be present in fresh, normal milk consisting of casein in combination with about 1.5% of CaO; *basic calcium casein* or *caseinate* is the compound consisting of casein in combination with about 2.5% of CaO. *Calcium Paracasein* or *paracaseinate* is the insoluble compound formed by the action of rennet in calcium casein; *paracasein* or *free paracasein* is the base-free or uncombined protein.” As Van Slyke rightly remarks, there is extreme confusion prevailing in the nomenclature of casein and any that is suggested must be purely provisional. It is advisable, therefore, when speaking of casein to describe exactly which substance is intended. Thus the word *casein* might be used to describe the protein actually present in milk, *rennase casein* and *acid casein* being used to describe the coagula produced by rennase and acid respectively, and *free casein* to describe the substance after separation and purification. In all cases where the actual nature of the substance meant is of importance, sufficient description should be given to it to identify it without possibility of error.

**The Nature of the Casein Present in Milk.**—As present in milk, casein is not in a state of true solution. This has been called “pseudo-solution,” which is, of course, merely another method of saying the same thing. The casein can be separated by intense centrifugal force or by filtering through porous porcelain as in the

<sup>1</sup> The last edition of this work.

ordinary Chamberlain filter. An ordinary laboratory filter candle of small size ( $110 \times 22$  mm.) will yield 20 c.c. of quite clear filtrate in twenty-four hours.

Richmond (*Dairy Chemistry*, 3rd Edit., p. 54) considers that in fresh milk casein is combined with calcium phosphate, calcium and sodium as  $\text{Ca}_3\text{Na}_2\text{H.Casein.2Ca}_3(\text{PO}_4)_2$ . When treated with acid this forms  $\text{Ca}_3\text{H}_4\text{.Casein.2Ca}_3(\text{PO}_4)_2$ , whilst when treated with rennase,  $\text{Ca}_3\text{H}_4\text{.Casein.2Ca}_3(\text{PO}_4)_2$  is formed. Many other suggestions have, however, been put forward, and it is not possible to state at present which, if any, is the correct one. It can be stated definitely, however, that the different precipitation reactions of casein under the varying conditions of each experiment are due to the different ways in which the casein is combined with bases. The best information now available seems to consider this substance to be a colloidal complex of calcium caseinate and calcium phosphate. See Rogers (*loc. cit.*) and Palmer (3rd Colloid Symposium Monograph, 1925, 112).

**Preparation of Casein.** (a) **Pure Casein.**—The preparation given below is that due to C. Crowther and H. Raistrick (*Biochem.*, 1916, **10**, 434). Similar methods have been devised by L. L. Van Slyke (*Amer. Chem. J.*, 1907, **38**, 398; Allen, 4th Ed., Vol. 8, p. 116) and by L. L. Van Slyke and A. W. Bosworth (*J. Biol. Chem.*, 1913, **14**, 203; Allen 4th Ed., Vol. 9, p. 595).

Freshly drawn milk (separated milk should be used and a super-centrifuge may be used with advantage) is diluted with about three times its volume of distilled water and heated to about  $40^\circ$ . Saturated potash-alum solution is then added in sufficient quantity to produce complete precipitation. After standing for half an hour the solution is decanted from the precipitate, which is then strained through muslin and washed three times by vigorously stirring with water. (The decanted solution, filtrate and washings are retained when it is required to isolate the proteins still remaining in solution.)

The precipitate is then suspended in distilled water, dissolved in the minimum amount of a 1% solution of caustic soda,<sup>1</sup> and the solution thoroughly extracted with ether and filtered. The filtered solution is then diluted with distilled water and the casein re-precipi-

<sup>1</sup> The use of caustic soda is undesirable, as it tends to split off nitrogen and phosphorus. When ammonia cannot be used, borax or other weak alkali might be advantageous. (Cf. Van Slyke, (*World's Dairy Congress*, 1923, 1149; *Chem. Age*, 1924, **32**, 163) who removes the last traces of mineral matter by electrophoresis.)

tated with 1% acetic acid, added with constant and thorough stirring. (L. L. Van Slyke carries out the first, as well as the subsequent precipitations, with acetic acid and dissolves the precipitate in dilute ammonia in place of caustic soda. The use of ammonia is not very suitable in those cases where nitrogen determinations are to be carried out on the product.) After settling, the supernatant liquid is syphoned off and the precipitate washed by sedimentation. This process of dissolving in alkali and re-precipitating is repeated at least five times. The final solution is filtered quite clear through filter paper pulp, diluted, and precipitated with the minimum amount of 1% hydrochloric acid. After washing and draining, the casein is dried by grinding with alcohol and then with ether, and finally, if necessary, *in vacuo* over sulphuric acid at a temperature not over 50°. (The ether should be pure and free from substances likely to combine with the protein molecule.) The final product should be a white dusty powder practically free from ash.

L. L. Van Slyke and A. W. Bosworth (*J. Biol. Chem.*, 1913, **14**, 203) remove calcium in the following way: The final solution in dilute ammonia is treated with 10 c.c. of strong ammonia (sp. gr., 0.88) and then with 20 c.c. of a saturated solution of ammonium oxalate, and the mixture allowed to stand for at least twelve hours. The precipitated calcium oxalate is removed by means of a centrifuge and subsequently by filtering through a close-texture filter paper (e. g. Whatman No. 5). The filtered solution is then treated with dilute hydrochloric acid (10 c.c. of 1.20 sp. gr. acid, diluted to 1 litre) until the casein is precipitated. The precipitate is washed with distilled water until free from chlorides filtered in a Buchner funnel and the preparation continued as above. By this means a preparation containing less than 0.1% of ash is obtained the amount of phosphorus present being about 0.70%, as compared with the usual figure of 0.85% found for those samples of casein from which the calcium is not removed.

(b) **Commercial Casein.**—On the industrial scale casein is usually precipitated by one of three methods—with acids such as sulphuric, hydrochloric or acetic, with rennet, or by spontaneous souring. A separated milk is always employed, and this should have as small a percentage of fat as possible. The reaction is carried out in pans made of iron or copper fitted with steam pipes so that the milk can be brought to the desired temperature. The precipitated curd is



washed with water which is then removed as far as possible in a centrifuge or filter press. The resulting cake is broken up and dried in a suitable form of drying oven, the temperature being carefully controlled so that the drying is carried out as rapidly as possible without the casein becoming discoloured. A description of a method for the preparation of "Grain-curd Casein" is given by W. M. Clarks and others. (*J. Ind. Eng. Chem.*, 1920, 12, 1163.)

**Properties of Casein.**—Pure casein is a fine, white, dusty powder having a sp. gr. of about 1.26. It is soluble in water only to the extent of about 0.1%. It is precipitated by mineral acids but is redissolved by excess. It is readily soluble in dilute alkalis; a solution resembling separated milk is obtained by dissolving casein in the requisite quantity of sodium phosphate solution together with a little calcium chloride. Casein behaves both as an acid and a base, although its acidic properties are somewhat the more pronounced. Its solutions in alkalis have a laevo rotation. Casein is also dissolved by alkaline earths and such alkaline salts as phosphates, borates, etc. From these alkaline solutions casein is re-precipitated on the addition of acids.

The molecular weight of casein has been variously estimated as from 1,000 to 200,000, although it is probably much nearer the latter than the former. A. W. Bosworth (*J. Soc. Chem. Ind.*, 1915, 34, 678) states that under the action of rennin one molecule of casein forms two molecules of paracasein and that the cleavage of nitrogen, phosphorus and calcium, noticed by Harden and Macallum (*Biochem. J.*, 1914, 8, 90), follows rather than accompanies this conversion. Cf. Cohn and Hendry, (*J. Gen. Physiol.*, 1923, 5, 521; 1924, 7, 45).

When milk is treated with acid or acid salts, the casein is precipitated as a heavy white solid in more or less flocculent form, depending on the conditions of treatment. When milk turns sour in the ordinary way, the lactic acid that is formed produces the same effect as the addition of any dilute acid; the precipitation or curdling occurs, at ordinary temperatures, when the acidity reaches 0.6 to 0.7%, expressed as lactic acid. When dilute artificial lactic acid is added directly to fresh milk, precipitation takes place at ordinary room temperature when the acidity reaches 0.57%. Increase of temperature enables a smaller amount of acid to precipitate casein; for example, milk curdling at room temperature with 0.55% of added

lactic acid, is precipitated with 0.35% at boiling temperature (Van Slyke).

When the casein of milk is precipitated by acids, the first action of the acid is combination with the calcium of the calcium casein, forming calcium lactate and free casein.

When more acid is present than is sufficient to combine with the calcium of the calcium casein, the insoluble phosphates of the milk are changed to mono-calcium phosphates, and further amounts of acid are more or less extensively absorbed by the base-free protein. These processes may, of course, occur simultaneously.

L. L. Van Slyke and D. D. Van Slyke (*Amer. Chem. J.*, 1907, **38**, 383) have furnished evidence that the action is one, not of chemical combination, but of adsorption. The amount of acid thus adsorbed varies (*a*) with the concentration of the acid, (*b*) with the duration of contact and degree of agitation until equilibrium is reached, (*c*) with the temperature, and (*d*) with the kind of acid. For example, the amount of hydrochloric acid adsorbed by casein varies from 8 to 50% of the acid originally in solution, according to the concentration of the acid; in the case of sulphuric acid, about 75% of the acid in solution is quickly adsorbed at ordinary room temperature. Lactic and acetic acids are taken up less completely. The acid thus removed from solution can be washed out of the casein by agitating with water.

Free casein dissolves easily in moderately dilute acids, more easily at higher temperatures, forming soluble compounds, which are either combinations of acid with the protein or decomposition products, depending on the concentration of the acid, the temperature, length of contact, etc. For example, solutions of acids of a concentration of *N*/125 dissolve casein quite readily at 25°. Little or no solution of casein occurs with solutions of acids not stronger than *N*/1,000 at 25°. The solvent action of the following acids is in this order, from strongest to weakest: Hydrochloric, lactic, sulphuric, acetic. The dissolved protein, at least in case of hydrochloric acid, contains more acid than the adsorption product. According to Long (*J. Amer. Chem. Soc.*, 1907, **29**, 1334) 1 grm. of free casein combines with about 7 c.c. of *N*/10 acid in the case of hydrochloric, hydrobromic, hydriodic, sulphuric and acetic to form soluble salt-like compounds. At higher temperatures, much more acid appears to combine, probably owing to hydrolysis.

According to the work of Laqueur and Sackur (*Hofmeister's Beiträge* 1902, 3, 193) casein obtained from cows' milk is 4 to 6 basic, and its equivalent weight about 1,135. According to Matthaiopoulos (*Z. anal. Chem.*, 1908, 47, 495) 1 grm. of free casein neutralises 8.8378 c.c. of *N*/10 sodium hydroxide, or 1 c.c. of *N*/10 sodium hydroxide combines with 0.11315 grm. of casein, making the equivalent weight 1131.5. According to Long (*J. Amer. Chem. Soc.*, 1906, 28, 372) the equivalent weight of casein in cows' milk is 1,124; in goat's milk 1,190. If the equivalent weight of casein is 1131.5, the molecular weight would be 4,526 if casein is tetrabasic, or 6,789 if casein is hexabasic. Cf. Cohn and Hendry (*supra*).

Casein is insoluble in sodium chloride, sulphate, nitrate, potassium chloride, or lithium nitrate solutions, but soluble in solutions of ammonium nitrate or acetate, sodium propionate, butyrate, valerianate, oxalate, rhodonate and potassium cyanide (Robertson, *J. Biol. Chem.*, 1907, 2, 317). It is soluble also in potassium oxalate and sodium fluoride (Arthus, *Compt. rend. soc. biol.*, 1893, 45, 327). Casein is also easily soluble in di- and tri-phosphate solutions (Hammarsten, *Maly's Jahresb.*, 1875, 5, 119).

Calcium casein is precipitated, apparently unchanged chemically, by saturating milk with common salt, ammonium sulphate, magnesium sulphate, etc. at ordinary temperatures. It is also precipitated by small amounts of alum, zinc sulphate and many other metallic salts. Calcium chloride and some other salts precipitate calcium casein in milk heated to 35 to 45°.

Heat alone under ordinary conditions, even at the boiling point of water, does not coagulate the calcium casein in milk. However, heated under pressure to 130–140°, casein salts are changed in their properties and casein itself is coagulated. The formation of a peculiar skin (haptogen membrane) on milk heated above 60° is largely due to the calcium casein of the milk and not, as was formerly supposed, to albumin. The skin itself contains practically all of the constituents of the milk and may be regarded as a kind of evaporated milk. On removing the membrane, a new layer is formed and, by removing these one after another, practically all of the milk can be transformed into the membrane condition. It appears to be due to surface evaporation. When milk is diluted with water and the surface covered with oil, no formation of skin takes place at boiling temperature. (Sembritzky, *Pflüger's Archiv.*, 1885, 37, 460;

Jensen and Plattner, *Landw. Jahrb. Schweiz.*, 1905). Cf. also W. Grimmer, *Chemie und Physiologie der Milch*, p. 67.

**Coagulation of Milk by Rennin.**—When a solution of rennin (*vide supra*) is added to milk, coagulation follows in a short time, the duration depending on the conditions under which the experiment is carried out. This subject was dealt with at length in the last edition of this work (Vol. VIII, p. 125 *et seq.*), and little further progress has been made in the elucidation of the changes taking place. (Cf. N. C. Wright, *Biochem. J.*, 1924, 18, 245.) H. M. Pavlovski and J. Zaykovski (*Fermentforsch.*, 1926, 8, 537) and A. de Dominicis and L. La Rotonda (*Annali. Chim. Appl.*, 1926, 16, 294). L. L. Van Slyke considers that the action of the enzyme takes place in three quite distinct stages: (1) change of calcium casein into calcium paracasein; (2) change of the calcium salts of the milk into soluble form; and (3) precipitation of uncoagulated calcium paracasein by the soluble calcium salts.

At about 38° one part of active rennin will coagulate some 10,000 parts of fresh milk, the time required at the same temperature being inversely proportional to the amount of rennin added. The greater the acidity of the milk the less rennin will be required. The optimum temperature is about 41°. Boiled milk is difficult and sometimes impossible to curdle. Cf. Palmer and Richardson, *Third Colloid Symposium Monograph*, 1925, 112; *J. Phys. Chem.*, 1929, 33, 557.

**The Testing of Rennet.**—For general purposes a simple test and some arbitrary standard are adopted. The Monrad test is as good as any. For this purpose 5 c.c. of the rennet extract are measured into a 50 c.c. flask and diluted to the mark, the pipette being washed out into the flask. 160 c.c. of milk are placed in a beaker and heated to about 29°–30° and a few small particles of straw dropped in. The rennet extract dilution is brought to the same temperature, and 5 c.c. are then quickly run into the milk, which is stirred at the same time. When the contents of the pipette are half out, a stop watch is started, and the milk, having been given a strong rotatory motion with the stirrer or thermometer, this last is withdrawn, and the watch stopped the moment the straw floats cease to move, indicating coagulation. In this manner, a certain dilution being taken as a standard, other extracts can be standardised by making the same dilution and comparing the times of coagulation. The relative strengths of the

extracts are then given in terms of the coagulation time. Other temperatures may be employed, but at 37° rennet is seriously influenced both by hydrion and hydroxyl ion concentration and since for cheese making, acidity is an all important factor, the lower temperature given above is better.

The time of rennet coagulation is influenced by many factors, especially acidity, and the conditions in comparative experiments must therefore be identical; see in this connection, Zimmermann, (*J. Ind. Eng. Chem.*, 1912, 4, 506).

For a very full and elaborately scientific method of testing rennet, see Van Dam. (*Landw. Versuchs. Stat.*, 1912, 78, 133; *Analyst*, 1912, 37, 564). The method is made of a standard character by the use of purified casein in a fixed low hydrogen ion concentration. (Van Slyke). Cf. also, Mattick, *J. Agric. Science*, 1930.

**The Composition of Casein.**—The elementary composition of uncombined casein is given by L. L. Van Slyke as:

	Per cent
Carbon.....	53.50
Oxygen.....	22.14
Nitrogen.....	15.80
Hydrogen.....	7.13
Phosphorus.....	0.71
Sulphur.....	0.72

Varying figures for casein obtained from different types of milk have been published, but these do not differ more than those obtained from cows' milk casein by different observers.

The amino-acid composition of casein has been found by F. W. Foreman (*Biochem. J.*, 1919, 13, 378-397) to be as follows: Glycine, 0.45; alanine, 1.85; valine, 7.93; leucine, 9.7; proline, 7.63; phenyl-alanine, 3.88; glutamic acid, 21.77; aspartic acid, 1.77; new syrups, 14.34; lysine, 7.62; histidine, 2.5; arginine, 3.81; tryptophane, 1.5; serine, 0.5; tyrosine, 4.5; hydroxyproline, 0.23; diaminotrihydroxydecanic acid, 0.75; ammonia, 1.61; sulphur, 0.76; phosphorus, 0.85; substances of peptide nature obtained on hydrolysis, 3.41; total 97.36%.

**Determination of Casein.**—A considerable number of methods for the determination of casein in milk have been proposed depending upon the use of different precipitating agents. All these methods do not give identical results, and the somewhat wide variations

found in published figures must be put down in some measure to this cause.

The official A. O. A. C. methods are as follows:

(This determination should be made while the milk is fresh, or nearly so. When it is not practicable to make this determination within 24 hours, add 1 part of formaldehyde to 2,500 parts of milk and keep in a cool place.)

**Method I.**—Place 10 grm. of the sample in a beaker with 90 c.c. of water at 40°–42° C. and add at once 1.5 c.c. of dilute acetic acid<sup>1</sup> (1 + 9). Stir, and allow to stand 3–5 minutes. Decant on a filter, wash by decantation two or three times with cold water, and transfer the precipitate to the filter. Wash once or twice on the filter. The filtrate should be clear, or very nearly so. If the first portions of the filtrate are not clear, repeat the filtration, after which complete the washing of the precipitate. Determine nitrogen in the washed precipitate and filter paper as usual, and multiply by 6.38 to obtain the equivalent of casein.

To a sample of milk that has been preserved, the acetic acid should be added in small portions, a few drops at a time, with stirring, and the addition should be continued until the liquid above the precipitate becomes clear, or very nearly so.

**Method II.**—To 10 grm. of the sample add 50 c.c. of water at 40° C. then 2 c.c. of potassium alum solution saturated at 40° C. or higher. Allow the precipitate to settle, transfer to a filter, and wash with cold water. Determine nitrogen in the precipitate and filter paper as usual.

Precipitation at approximately the iso-electric point has been suggested by H. C. Waterman (*J. A. O. A. C.*, 1927, 10, 259), who avoids the tedious washing of the precipitate by the determination of the total nitrogen in the milk and in the serum after precipitation. His method is as follows:

**Reagent:** Pipette 250 c.c. of normal acetic acid into a 1,000 c.c. flask. Add 125 c.c. of normal carbon-dioxide-free sodium hydroxide. Make up to 1,000 c.c. with carbon-dioxide-free distilled water and mix thoroughly.

**Determination:** Pipette 20 c.c. of the sample into a 100 c.c. flask. Add 50 c.c. of the reagent, mix, make up to volume with distilled

<sup>1</sup> "Hoppe-Seyler recommends the passage of carbon dioxide into the solution to complete the precipitation."

water, and shake well. Set the flask in hot water ( $50^{\circ}$ – $60^{\circ}$ , not over  $60^{\circ}$ ) and leave for 15 minutes. Cool to room temperature and filter. Use a double folded paper, returning the filtrate once or twice to the filter; then filter once through a hardened paper. Determine nitrogen (A) in 50. c.c. of the clear filtrate, and determine total nitrogen (B) in 10 c.c. of the milk.  $6.38 \times (B - A) =$  casein in 10 c.c. of the milk. Report grm. of casein per 100 c.c. of milk, or divide the grm. per 100 c.c. by the density of the milk, and report as percentage by weight.

In Leffmann and Beam's modification of Sebelein's method 20 grm. of milk are diluted with twice its bulk of a saturated solution of magnesium sulphate (free from sodium sulphate) solid magnesium sulphate is added to the point of saturation, and the whole made up to a definite volume. The soluble nitrogen is determined in an aliquot portion of the serum, and this is subtracted from the total nitrogen of the milk, to give the nitrogen as casein.

There is a somewhat urgent need for a standard process for the determination of casein which will be both rapid (all processes which involve the washing of precipitated casein are tedious and likely to give erroneous results) and accurate. The acetic acid process of the A. O. A. C. seems to be the one now most frequently used, but the modification due to Waterman (*vide supra*) and the extension of this described below would appear to be an improvement and deserves an extended trial.

G. M. Moir (*Analyst*, 1931, 56, 14) has extended the method of Waterman as described below; this must be looked upon as the best available method.

Into a weighed covered beaker (100–150 ml.) pipette 10 ml. of the well-mixed sample and weigh again quickly. Dilute the milk with about 50 ml. of distilled water which has been first warmed to within  $40^{\circ}$ – $42^{\circ}$ . Add at once 1.5 ml. of 1.67 *N* (10%) acetic acid, and then stir gently by rotating the stirring rod four times in the beaker. Excessive stirring is to be avoided. After allowing the mixture to stand about 20 minutes to attain equilibrium add 4.5 ml. of 0.25 *N* sodium acetate solution, and, after stirring gently, leave for at least an hour. Filter through a 9 cm. No. 42 Whatman filter, which it is desirable to fold in the fluted way to facilitate the operation. Wash the precipitate with distilled water three times by decantation and follow by two further washings in which the precipitate is

macerated and transferred to the paper. Finally, rinse the rim of the filter paper with a fine stream of water. The filtration and the washing should be carried out without interruption, and subsequently the casein adhering to the beaker and stirring rod should not be allowed to dry before being washed out with the sulphuric acid that is required for the Kjeldahl digestion. For this purpose about 20 ml. of water are placed in the beaker and about 5-7 ml. of the strong acid carefully poured down the side. The heat generated by gentle mixing helps to dissolve the casein, which is usually completely removed after three such treatments. The filter paper and casein are added to the Kjeldahl flask into which the washings from the beaker have been poured; the usual quantity of sodium or potassium sulphate and a trace of copper sulphate are added before commencing to heat the digestion flask. It is desirable to evaporate the water with a small flame, as frothing is liable to occur just before the last of the water is boiled off. During digestion some of the acids from the fat condense upon the neck of the flask and may subsequently cause trouble by frothing during the distillation. These can be destroyed if the flask is allowed to cool when digestion is nearly complete, and about 50 ml. of water carefully added and mixed with the contents. The fatty material is washed down by the condensation of steam during evaporation of the water. The determination is completed in the usual way.

Volumetric methods for the determination of casein have been suggested by various workers, e. g. G. Deniges (*Bull. Soc. Chim.*, 1896, **15**, 1116). E. B. Hart (*J. Biol. Chem.*, 1909, **6**, 445) L. L. Van Slyke and A. W. Bosworth (*J. Ind. Eng. Chem.*, 1909, **1**, 768) and C. B. Hersey (*J. Ind. Eng. Chem.*, 1916, **8**, 335), who considers that Hart's method is quicker than that of Van Slyke and Bosworth and that it gives results nearer to those given by the Kjeldahl method. Van Slyke and Bosworth's method is as follows. Twenty c.c. of milk are run into a 200 c.c. graduated flask, phenolphthalein is added, and then  $N/10$  sodium hydroxide solution in small quantities at a time, with constant and vigorous shaking, until a faint but distinct pink tint is permanent. Excess is to be avoided. The solution, which should be at a temperature of from 18-24°, is then treated with  $N/10$  acetic acid in quantities of about 5 c.c. at a time up to 25 c.c., and thereafter in quantities of 1 c.c., until, on standing, the casein separates promptly in large white flakes. The amount of



acid added is noted as A. The liquid is then diluted to the mark and the whole vigorously shaken. It is then filtered, when, if the right amount of acid has been added, the filtrate will be practically clear and the rate of filtration will be reasonably quick. 100 c.c. of the filtrate are then titrated with  $N/10$  sodium hydroxide until a faint but permanent pink tint is obtained. The quantity of hydroxide added is noted as B.

$$\text{Percentage of casein} = \left( \frac{A}{2} - B \right) \times 1.0964$$

The process is not satisfactory with milks which are sufficiently sour to curdle on boiling. When the determination cannot be carried out at once the milk may be preserved for a week longer with about 1 part in 1,000 of mercuric chloride.

A refractometric method for casein is due to Robertson (*J. Ind. Eng. Chem.*, 1909, 1, 723). A method similar to the aldehyde figure (*vide supra*) has been proposed by Walker (*J. Ind. Eng. Chem.*, 1914, 6, 131).

The following method for the determination of casein in chocolate has been proposed as a tentative method by the A. O. A. C. Weigh 10 grm. of the chocolate into a 500 c.c. Erlenmeyer flask and add 250 c.c. of 1% sodium oxalate solution. Heat to boiling and boil gently for a few minutes; then cool, add 5 grm. of magnesium carbonate, and filter. Determine nitrogen in 50 c.c. of this filtrate. Pipette 100 c.c. of the filtrate into a 200 c.c. volumetric flask and dilute almost to the mark with water. Precipitate the casein by the addition of 2 c.c. of glacial acetic acid or 1 c.c. of concentrated sulphuric acid. Make up to volume, shake, filter, and determine nitrogen in 100 c.c. of the filtrate. The difference between the two nitrogen determinations gives the nitrogen derived from the casein which, multiplied by 6.38, gives the quantity of casein present in 2 grams of the sample.

### THE AMOUNT OF CASEIN PRESENT IN MILK

As the casein accounts for the major portion of the total proteins in milk, the remarks made under this latter heading above will apply to a large extent to the casein alone. The average amount present in cows' milk has been reported as varying from 2.5% (Van Slyke) to 3.4% (Oliver). Richmond gives the figure for English cattle

as 3.0%, whilst Tocher, for Scottish cattle, finds 2.43%. It is a significant fact that Tocher finds considerably more albumin than Richmond, and it is quite likely that these differences are due, at least in part, to different methods of separation.<sup>1</sup> In individual cows Tocher found the casein to vary from 1.64% to 4.14% whilst Van Slyke found 1.59% to 4.49% with, of course, much narrower limits for herds, the milk from a herd of cows will usually yield between 2.6% and 3.1% of casein. In colostrum the amount of casein (about 4.5%) is usually higher than in milk but this does not account for the great difference in the amount of total proteins (*vide infra*).

### COMMERCIAL CASEIN

Casein prepared on the commercial scale is not pure, the amount of ash present depending upon the method of preparation. Rennin prepared casein contains the most ash, self-soured casein the least.

A. Burr (*J. Soc. Chem. Ind.*, 1910, 29, 1327) gives the average composition as:

Water.....	10.38
Fat.....	1.89
Casein.....	79.45
Ash.....	6.51
Other ingredients.....	1.77
	100.00

and for caseins prepared by himself.

	4 samples of acid casein	4 samples of rennet casein (paracasein)	15 samples of curd precipi- tated by rennet and acid	Mean
Water ..	5.55 - 8.65	0.6 - 2.97	73.08-80.03	76.70
Dry substance.....	91.35 - 94.45	97.03-99.40	19.97-26.92	23.39
Fat ..	0.05- 0.075	0.08- 0.55	0.36- 1.10	0.66
Ash ..	0.0	5.0 - 8.55	1.18- 2.18	1.59
Nitrogen content of casein, fat- and ash-free.....		15.58-15.63	15.64-15.74	
Conversion factor of casein, fat and ash free.....		6.40- 6.41	6.35- 6.40	

The United States Specification for commercial casein is (*J. Ind. Eng. Chem.*, 1919, 11, 1019): Colour, light cream or white; odour,

<sup>1</sup> Tocher allowed his casein precipitate to stand over night in contact with the acid. This is likely to have dissolved some casein and thus increased the amount of albumin.

very slight; water, not more than 10%; fat, not more than 1%; ash, not more than 4%; nitrogen not less than 14.25%, (*i. e.* 91% of casein); acidity, not more than 10.5 c.c. of *N*/10 alkali per gram. H. Ulex (*Chem. Ztg.*, 1925, **49**, 641) considers that a good casein may contain as much as 2.5% of fat, cf. Hopfner and Burmeister (*Chem. Ztg.*, 1912, **36**, 1053).

### METHODS OF ANALYSIS OF COMMERCIAL CASEIN<sup>1</sup>

In the examination of commercial caseins the usual determinations are moisture, ash, fat, nitrogen and acidity. Sugar, phosphorus and calcium are sometimes required. The colour, odour, degree of aggregation, fineness and general appearance should be recorded.

**Moisture.**—This may be determined by drying 1 or 2 gm. at 100°, taking care that decomposition does not take place, or by drying *in vacuo* at 70°–80°. Where much free acid is present, particularly mineral acid, decomposition may be caused and a high result for moisture indicated. Where much browning takes place on heating the amount of water should be checked by difference. By taking frequent weighings and observing the loss in weight, the loss due to decomposition, which will then be fairly constant in a given time, may be distinguished from loss due to moisture.

**Ash.**—By ignition at a dull red heat preferably in an electric muffle. R. H. Shaw (*J. Ind. Eng. Chem.*, 1920, **12**, 1168) treats 3 gm. of casein with 5 c.c. of calcium acetate solution, ignites at a low red heat, and subtracts the weight of CaO obtained from the calcium acetate from the total weight recorded.

**Fat.**—Direct extraction with ether in a Soxhlet tube, even after grinding with sand, usually gives low results. There has been considerable discussion as to whether the Röse-Gottlieb or the Werner-Schmid process gives the more correct results. See D. A. Gangolli and A. N. Meldrum (*J. Soc. Chem. Ind.*, 1921, **40**, 746A), G. T. Bray and F. Major (*J. Soc. Chem. Ind.*, 1923, **42**, 106T), W. Hoepfner and K. Jaudas (*Chem. Ztg.*, 1925, **49**, 281; 1926, **50**, 325), and H. Ulex (*Ibid.*, 1925, **49**, 641). Probably the Werner-Schmid process is the better. 5 gm. of casein are intimately mixed with 10 c.c. of water, 20 c.c. of conc. hydrochloric acid added, and the whole heated in the boiling water bath for forty minutes. After

<sup>1</sup> It is not easy to correlate the result of analysis of casein with its suitability for particular purposes. See Sutermeister. "Casein" p. 195.

cooling, the fat is extracted with ether in the usual way, and the dried fat re-extracted with petroleum spirit, to correct for any non-fatty solids extracted by the wet ether.

**Nitrogen.**—This is determined by the Kjeldahl process in the usual way. Nitrogen  $\times 6.38$  gives casein (or, rather, total proteins).

**Acidity.**—1 grm. is dissolved in 25 c.c. of  $N/10$  sodium hydroxide solution, the solution diluted to 100 c.c. and titrated back with  $N/10$  hydrochloric acid, with phenolphthalein as indicator. The acidity is expressed as c.c. of  $N/10$  alkali used for 1 grm. of casein. The American specifications state that the calculation should be made on the dry, fat-free and ash-free substance. Methods for the determination of real acidity are given by Hoepfner and Jaudas and by Ulex (*vide supra*).

**Other Determinations.**—Sugar may be determined by shaking 10 grm. for 4 hours with 250 c.c. of 50% alcohol, allowing the mixture to settle, and using 100 c.c. for the determination. Phosphorus is determined by the usual methods for fertilisers, and calcium in the ash after digestion of the casein with *aqua regia* (R. H. Shaw).

H. F. Zoller (*J. Ind. Eng. Chem.*, 1920, **12**, 1171) has suggested that the borax solubility test should be standardised as follows: The viscosity of casein in borax solution is at a maximum at a hydrogen ion concentration of  $pH = 8.15$ , whilst at  $pH = 8.09$  to  $9.1$  the viscosity is lower, but constant, owing to the buffer effect of borax in this region. The concentration of casein chosen for the improved casein-borax test is about 12% of true casein in  $M/5$  sodium borate solution. (See Mummery and Bishop, *Analyst*, 1930, **55**, 367).

E. Sutermeister (*J. Soc. Chem. Ind.*, 1914, 588) has published the following test for the determination of the strength of casein for use in coated papers:

For testing the solubility, 50 grm. of casein, ground to pass through a 20-mesh sieve, are mixed with 200 c.c. of water and 7.5 grm. of borax; the mixture is heated on a water bath and stirred until the mixture becomes homogeneous. The temperature should not exceed  $82^{\circ}$ , nor the time of stirring 10 minutes. Some samples of casein require slightly more borax, some show a small quantity of white flaky residue. For determining the strength of the casein, a direct coating-test should be made: 50 grm. of casein are placed in a tared beaker with 190–200 c.c. of water and the necessary amount

of borax to effect complete solution. When dissolved, the weight of the solution is made up to 250 gm. 100 gm. of fine clay, dried at 100°, are ground with 70 c.c. of water in a separate vessel, the mixture is weighed together with a copper spatula, and 30 gm. of the casein solution (= 6 gm. of casein to 100 gm. of dry clay) are stirred in. A sheet of paper is evenly coated on a level surface with this mixture, a curved steel scraper being used; the mixture is weighed again, and more casein added to bring the proportion of casein up to 7 per 100 of clay. Successive coatings are thus made with various mixtures up to 11% of casein. When all are dry, portions are selected which are equally and evenly coated, and short sticks of sealing wax are stuck vertically by melting upon the coated surface. These are pulled off, when cold, with a vertical pull, and that proportion of casein is said to be strong enough which shows an even layer of paper fibres adhering to the coating pulled off on the end of the wax. For standardising this test, it is necessary to work with standard samples both of clay and paper, large quantities being stocked, since variations in the quality of these cause variations in the absolute results of the test.

## COMMERCIAL APPLICATIONS OF CASEIN

1. **Foodstuffs.**—Many proprietary foods prepared from milk proteins (for convenience those prepared from lactalbumin will be dealt with here) have been placed on the market. They may, in general, be examined by the methods given under commercial casein above.

*Lactarine.*—A secret preparation containing about 10% of water, 0.4% of fat, 78% of casein, 7.8% of carbohydrates and 3.8% of ash. It is a fine white powder soluble in water.

*Galactogen.*—Another secret preparation containing about 4% of fat, 70% of proteins and 2% of phosphoric acid.

*Sanagen.*—A white soluble powder containing about 5% of sodium glycerophosphate with about 77% of total proteins.

*Sanatogen.*—A white soluble powder containing about 5% of sodium glycerophosphate and about 80% of total proteins.

*Plasmon.*—A yellowish white powder containing about 80% of milk proteins, 5% of sodium carbonate and 5% of ash and carbohydrates.

Other milk protein preparations are Dr. Reigel's milk protein, Nutrium, Eucasein and Nutrose. Cf. Aufrecht, *Chem. Ztg.*, 1900, **24**, 538.

*Artificial Milks.*—These have been prepared from entirely vegetable products, the most popular being the soya bean. Many patents have been taken out the following being typical of the method involved (*J. Soc. Chem. Ind.*, 1915, **34**, 196; *Eng. Pat.* 24,572, of 29.10.13). For the production of about 100 litres of the milk, 15 kilos. of soya beans ground to a fine meal are mixed at 90° C. with 100 litres of previously boiled water and 20 gm. of potassium phosphate, this temperature being maintained, with constant stirring, for about  $\frac{3}{4}$  hour. After the liquor has been passed through a filter press, the oil, which imparts an unpleasant taste to the liquid, is eliminated by means of a cream separator. Sesame or other tasteless oil (about 3.6 kilos.) and butyric and other acids, such as are usually found in cream, are next emulsified with the milk, and 1,875 gm. of dextrose, 2,500 gm. of dextrin, and 625 gm. of sucrose; or 1,500 gm. of maltose, 2,500 gm. of dextrin, and 1,000 gm. of sucrose; or 1,250 gm. of lactose, 1,000 gm. of maltose, 2,500 gm. of dextrin, and 250 gm. of sucrose are added. The milk is mixed with 100–130 gm. of sodium bicarbonate, 50–70 gm. of sodium chloride, and 50 gm. of citric acid, and, in order to impart a characteristic taste and aroma, and to cause it to separate into curds and whey at the end of a certain time, it is inoculated with a "cream starter" and allowed to stand until 100 c.c. require 4–5–7.5 c.c. of *N*/10 alkali for neutralisation. The milk is subsequently pasteurised, cooled to atmospheric temperature, and passed through a filter cloth, being then bottled for use.

The composition of soya bean milk is given by E. Remy (*J. Soc. Chem. Ind.*, 1922, 681A) as: Water, 88.93% dry matter, 11.07%; fat, 3.06%; non-fatty solids, 8.01%; protein, 2.96%; starch, 0.57%; glucose, 2.48%; mineral matter, 0.63%; alkalinity of ash, 6.44 c.c. of *N*/1 acid.

The detection of soya bean in milk is carried out by K. Nakayasu (*J. Pharm. Soc. Japan*, 1921, **476**, 880) by treating it with 28% caustic potash solution, when, in the presence of soya bean, a yellow colour is produced.

**2. Medicinal Products.**—Many compounds of casein have been produced for use as medicinal products. Many advantages for

these have been claimed, among which are non-irritation of the stomach, and, in some cases, the fact that the compound may be insoluble in the acid fluid of the stomach but soluble in the alkaline fluid of the intestines.

Compounds of casein have been prepared with glycerophosphates (Eng. Pat. 23,097 of 10.10.12, (*J. Soc. Chem. Ind.*, 1913, **32**, 989), silver (W. Pauli and J. Matula *Biochem Z.*, 1917, **80**, 187), tannin (Eng. Pat. 114,158 of 12.3.18; *J. Soc. Chem. Ind.*, 1919, **38**, 304A) and aluminum (Ger. Pat. 312,222 of 28.10.17; *J. Soc. Chem. Ind.*, 1919, **38**, 925A; Ger. Pat. 312702 of 20.1.18; *J. Soc. Chem. Ind.*, 1920, **39**, 427A. Similar preparations have been made containing salicylates, alkaloids, mercury, iron, arsenic, etc.

**3. Casein Paints.**—Casein paints are, as a rule, mixtures of casein, slaked lime and pigment, although in some cases the casein is mixed with the usual constituents of oil paint. The correct proportion of the ingredients is important, so that the paint shall be washable on the one hand, and not too brittle so that it cracks and peels off, on the other. For full details on this and other industrial applications of casein, reference should be made to "Casein" by Robert Scherer, London. Scott, Greenwood & Sons, 1921; or "Casein" by E. Sutermeister, New York, Chemical Catalog Company, 1927.

**4. Casein Adhesives.**—For use in adhesives casein must be practically free from fat, quite free from acid and from soluble calcium salts. For this reason it is not infrequently prepared for this purpose by precipitation with tannin-containing barks, etc. The casein is then formed into a paste (casein cement) or a liquid (casein glue) by means of borax, lime, soda or many of the alkalis and alkaline earths or their salts.

**5. Casein Plastics.**—When casein is suitably treated or mixed with various organic or inorganic substances it is converted into plastic masses which can be moulded and, when dried, turned in a lathe. By the addition of suitable stains various colours can be obtained and imitations of many materials produced. Of the various additions which have been suggested, acetone and other ketones, aniline and other amines, formaldehyde, cuprammonium compounds, hydrogen peroxide, sodium silicate, alum, aluminium acetate, karaya gum and viscose may be mentioned. *Galalith* and *Lactoform* are patent preparations, prepared from casein, which may be used as substitutes for celluloid.

**6. Other Industrial Uses.**—Casein is used in a variety of ways, the number of which is increasing rapidly. It has been found to be valuable in the production of coated papers, also in the textile industry for finishing purposes, and for the treatment of fabrics for colour printing. Casein is also used for the preparation of grease-proof containers, boot-polish, canvas, ointments and even soap.

## ALBUMIN

The albumin present in milk (lactalbumin) is, when prepared in a state of purity, a white powder, amorphous and tasteless. In aqueous solution about 90–95% is precipitated on heating to 70°. It can be prepared in a crystalline condition if its aqueous solution is saturated with pure magnesium sulphate, diluted with an equal bulk of water, a little acetic acid added, and the whole allowed to stand.

Lactalbumin is not precipitated by means of saturated magnesium sulphate solution, but it is precipitated from such a solution by the addition of acetic acid, the precipitate being dissolved when the solution is neutralised. It is precipitated by saturating its solution with ammonium<sup>1</sup> or sodium sulphate and by a solution of tannin or phosphotungstic acid.

Lactalbumin has the following elementary composition:

Carbon, 52.51; hydrogen, 7.10; nitrogen, 15.43; sulphur, 1.02; and oxygen, 23.04.

The main differences from casein are the absence of phosphorus and the considerably larger amount of sulphur. D. B. Jones and C. O. Johns (*J. Biol. Chem.*, 1921, **48**, 347) have returned the amino acids obtained on hydrolysis as: glycine 0.37%, alanine 2.41%, valine 3.30%, leucine 14.03%, proline 3.76%, phenylalanine 1.25%, aspartic acid 0.30%, glutamic acid 12.89%, hydroxyglutamic acid 10.00%, serine 1.76%, and tyrosine 1.95%.

Crowther and Raistrick (*vide infra*) have shown that lactalbumin from either milk or colostrum is very different in composition from serum albumin from ox blood.

The following recent publications on milk albumin or similar substances are of interest: "The Density of Albumin Solution,"

<sup>1</sup> For the coagulation temperatures of albumin in solutions of ammonium sulphate see K. Micko (*Z. Unters. Nahr. Genussm.*, 1911, **21**, 646). For the mechanism of the coagulation see W. D. Bancroft (*J. Physical Chem.*, 1915, **19**, 340) and M. A. Rakusin and A. Rosenfeld (*Z. Unters. Nahr. Genussm.*, 1925, **49**, 38).



M. A. Rakusin and G. D. Flieher; *J. Gen. Physiol.*, 1923, **5**, 383. "The Composition of Albumin"; I. M. Kolthoff, *Chem. Weekblad*, 1925, **22**, 489, and H. R. Kruyt, *Ibid.*, p. 473.

**The Preparation of Pure Albumin.**—C. Crowther and H. Raistrick (*Biochem. J.*, 1916, **10**, 466) prepared pure lactalbumin by the method of Pinkus (*J. Physiol.*, 1901, **27**, 57). The combined filtrates from the globulin preparations from milk (*vide infra*) are heated to 40°, saturated with pure anhydrous sodium sulphate (free from chloride) and allowed to stand at 40° for a few hours. The precipitated albumin is then filtered off through a jacketed filter at 40°, crystallisation of the salt being prevented. The precipitate is dissolved in cold water, the solution filtered, and the filtrate saturated with magnesium sulphate. Any precipitate is removed by filtration, and the filtrate again saturated with anhydrous sodium sulphate at 40°. This process is repeated four times, or once more after saturation with magnesium sulphate gives no precipitate. The final preparation is dissolved in water, the solution filtered, and the albumin precipitated with alcohol and dried with alcohol and ether at a low temperature. For an alternative method of preparation see H. F. Woodman. (*Biochem. J.*, 1921, **15**, 187.)

J. Reitstotter (*Kolloid Z.*, 1923, **32**, 47) states that solutions of albumin cannot be submitted to prolonged dialysis without permanent changes taking place in the material. For the change of albumin to globulin see "Globulin" below.

**Determination of Albumin.—Method I.**—The official method of the American A. O. A. C. is as follows: Exactly neutralise the filtrate obtained from the Official Method (I) for the determination of casein with 10% sodium hydroxide solution, add 0.3 c.c. of dilute acetic acid (1 + 9), and heat on a steam bath until the albumin is completely precipitated. Collect the precipitate on a filter; wash with cold water; determine the nitrogen as usual, and multiply by 6.38 to obtain the equivalent of albumin.

**Method II.**—To the filtrate obtained from the casein by the Official Method (II) add 0.3 c.c. of dilute acetic acid (1 + 9), boil until the albumin is completely precipitated, and proceed as in the Official Method (I) for albumin.

The precipitation of the albumin may also be carried out by boiling the filtrate from the casein or by precipitation with tannic acid or with phosphotungstic acid. Nitrogen may be determined in the

precipitate in the usual way. It is doubtful whether these methods give identical results; this point is discussed further below. W. Grimmer C. Kurtenacker and R. Berg (*Biochem. Z.*, 1923, **137**, 465) state that the serum proteins are, as a rule, incompletely precipitated by heat, acids or salts, and that the residual nitrogen in the serum after precipitating with phosphotungstic acid is 0.027%, and after precipitating with tannin is 0.028%. It is not at all certain, however, that this nitrogen is albuminoid. (Cf. G. M. Moir, *Analyst*, 1931, **56**, April.)

M. Claudius has suggested an ingenious method for the determination of albumin (Ger. Pat. 253,978 of 21.12.11; *J. Soc. Chem. Ind.*, 1913, **32**, 164). The precipitant is a 2% aqueous solution of trichloroacetic acid to which is added 0.5% of tannic acid and 0.1% of acid magenta. After the albumin is precipitated the colour of the filtrate is compared with that of the original reagent, the diminution in intensity being a measure of the quantity of albumin precipitated.

For other methods of determination see P. A. Kober, (*J. Amer. Chem. Soc.*, 1913, **35**, 1585); E. Reigler, (*Z. anal. Chem.*, 1914, **53**, 242) L. Lewin, (*Ann. Chim. anal.*, 1914, **19**, 281). W. O. Walker and A. F. G. Cadenhead, (*J. Ind. Eng. Chem.*, 1914, **6**, 573) and P. Cristol and M. Simonnet, (*J. Pharm. Chim.*, 1922, **26**, 829).

**The Amount of Albumin in Milk.**—The figures given by different observers for the average amount of albumin present in milk differ somewhat widely. They may, however, be more or less sharply divided into two groups ranging round 0.4% and 0.7%, respectively. The figures of Richmond, Oliver and Willoughby are in the former group, those of Tocher, Van Slyke, Babcock and Fleischmann in the latter. It would appear, therefore, quite probable that at least a portion of the variations found is due to differences in the methods of separation. In the present state of our knowledge, therefore, it is not possible to state with certainty the average amount of albumin present, but some tentative experiments of the writer would suggest that 0.4% is nearer the truth than 0.7%. The subject is being further investigated.

In the milk of 474 individual cows J. F. Tocher found the albumin to vary from 0.45% to 1.35%. These figures may be high, but they are at least comparable among themselves and suggest the amount of variation likely to occur.

## GLOBULIN

Lactoglobulin is coagulated when its solution is heated at  $72^{\circ}$ . It is not coagulated by rennase, and it is soluble in acidified solutions of sodium chloride. It is precipitated by neutral sulphates, tannic acid, etc., and is therefore usually returned along with the albumin. Not more than traces occur in normal milk (Crowther and Raistrick isolated 0.03%), but in colostrum large quantities, of the order of several units per cent, are frequently present. There are apparently two modifications, known respectively as eu-globulin and pseudo-globulin. The former is soluble in a 0.6% solution of sodium chloride solution, but insoluble in a 0.06% solution of this substance. The latter is soluble in water, but precipitated by the addition of alcohol to its aqueous solution.

S. Rusznyak (*Biochem. Z.*, 1923, **140**, 179) states that globulin can be converted into albumin under certain conditions, and that the reverse change can also take place. K. Gutzeit (*Z. ges. exp. Med.*, 1924, **39**, 397) states that albumin can change into globulin in 2 to 4 days (Cf. O. Arnd and E. A. Hafner *Biochem. Z.*, 1926, **1167**, 440), but this is, at least, doubtful.

T. B. Osborne, A. J. Wakeman, C. S. Leavenworth and O. L. Nolan (*J. Biol. Chem.*, 1918, **33**, 7) consider that, since the phosphorus content of lactoglobulin is reduced from 2.2% to 0.24% by treatment with alcohol, globulin is really lecitalbumin, albumin being almost free from phosphorus. T. B. Osborne and A. J. Wakeman (*J. Biol. Chem.*, 1916, **28**, 1) find that 1 litre of milk contains 27 mgrm. of phosphatides, and that there is much more phosphatide in albumin than in casein.

Crowther and Raistrick have shown that lactoglobulin from either colostrum or milk is very closely allied to, and is probably identical with, the serum-globulin of ox blood. This is confirmed by H. E. Woodman.

**Preparation of Globulin.**—The filtrate from the preparation of casein (*vide supra*) is neutralised with dilute soda and saturated with anhydrous magnesium sulphate. The precipitated globulin is filtered off on a large Buchner funnel. The precipitate is dissolved in a dilute solution of magnesium sulphate, filtered through pulp, saturated with magnesium sulphate, and the globulin filtered off as before. The precipitation is repeated four times, the filtrates being

reserved each time (if required) for the preparation of albumin. The final solution is dialysed, first in running water and then in relays of distilled water, until free from sulphates. The eu-globulin is rendered insoluble and is then filtered from the solution of pseudo-globulin, dissolved in a 0.6% solution of sodium chloride, filtered and re-precipitated by diluting with ten times its volume of distilled water. The precipitate is allowed to settle, washed by decantation first with distilled water, and afterwards with alcohol and then ether. The pseudo-globulin is precipitated from its aqueous solution by means of alcohol, filtered, and the precipitate washed with alcohol and ether. (Crowther and Raistrick, *loc. cit.*; see also M. E. Woodman, *loc. cit.*)

**Determination of Globulin.**—No simple method has been suggested for the determination of globulin in milk. The amount is, however, so small that the determination is not usually required, and, unless a very large quantity of milk is used, it is not likely to be carried out with any near approach to accuracy. In cases where this determination is required a process could be worked out based on the method of separation of Crowther and Raistrick given above. (Cf. K. Gutzeit, *J. Chem. Soc.*, 1924, *A*, ii, 636.) (Cf. G. M. Moir, *Analyst*, 1931, **56**, 220.)

I am greatly indebted to Dr. G. M. Moir, Pedler Research Scholar of the Institute of Chemistry of Great Britain and Ireland who, having read this section in manuscript, has made several valuable suggestions which have been incorporated.

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# MILK

BY HENRY LEFFMANN

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Milk is the nutrient secretion of nursing mammalia, and in this article, unless otherwise stated, cows' milk is to be understood.

Normal milk is a white or pale yellow opaque fluid, which coagulates in the stomach of the suckling young. Its formation from the blood of the parent is not fully understood but it necessarily has the same osmotic pressure as the blood. For this reason the depression of freezing point ( $\Delta = 0.55^{\circ} \text{C.}$ ) is a physical constant which, like that of blood, varies very little in the fresh milk of a healthy cow.

The specific gravity varies from 1.028 to 1.035. The reaction of fresh milk is approximately equal to  $\text{pH} = 6.65$ , but milk from cows which are drying off or have some bacterial infection of one or more quarters may have a  $\text{pH}$  of over 7 and give a purple colour when tested with yellow brom-cresol purple papers. This invasion of the udder is, however, abnormal the normal bacterial flora of the udder being small and producing little change in the milk.

Milk is an excellent medium for the growth of micro-organisms and unless great precaution is taken becomes infected especially with lactic acid producing organisms.

Milk is a very complex physiological liquid of varying composition. It always contains the following ingredients, which though frequently considered as separate entities in milk analysis, are themselves complex and interrelated with other components in fresh milk.

*Fat*.—The fat can be decomposed into glycerol and some nine different fatty acids (see Butter, Vol. II); it is also associated with lecithin, cholesterol and with the vitamins A and D.

Milk-fat exists in suspension in the form of globules, mostly ranging within the limits of 0.0024 mm.—0.0046 mm., average about

0.0037 mm., but globules above and below these limits are sometimes found. These globules exist under conditions that interfere with spontaneous coalescence; hence masses of fat are not readily formed and even high centrifuging will not develop a clear fatty layer. The globules being specifically lighter than the liquid in which they are suspended, tend to rise when the milk is allowed to stand and to form a layer much richer in fat but still showing no marked coalescence. This layer may be removed by direct skimming, or better by centrifugal force. The remaining liquid is termed "skim milk." By the use of separators almost the whole of the fat may be removed. The product termed "separated milk" is still moderately opaque, although containing only traces of fat. The fat of milk may be made to coalesce by mechanical agitation; this method (churning) yields it as a soft mass (butter) and leaves an opaque liquid (buttermilk). As cream has often stood for a time before churning, decomposition has set in, and the buttermilk is therefore slightly acid and the proteins slightly hydrolysed.

*The carbohydrate* of milk, lactose, is in complete solution. Lactose is fully described in Vol. I.

*Citric acid* is also a normal constituent of milk.

*The proteins* consist chiefly of casein with albumin and globulin and traces of other nitrogenous substances.

*Casein* is the most abundant protein of milk and is the substance that produces the bulk of the curd. In most methods of curdling, especially those with acids or enzymes, this protein is hydrolysed, a part being precipitated and a part remaining in solution.

When the milk is boiled in the open air a scum of casein and calcium phosphate is formed, but this is due to surface evaporation, not to coagulation.

The liquid filtered from the curd is termed "whey." It contains under most conditions of precipitation a notable amount of proteins.

Albumin, being coagulated by boiling, is found in an insoluble form in milk that has been heated to or near 100°.

Globulin is present in very small amount in normal milk and does not need special description.

*Mineral Matter.*—Among the mineral constituents of milk are found the following metals:—potassium, sodium, calcium, magnesium and traces of iron, copper and zinc. The non-metallic elements include phosphorus, chlorine, sulphur and iodine. Some of

these ash constituents, as they are called, exist in milk not only as salts but are also in combination with the proteins.

In addition to the fat-soluble vitamins mentioned above, other vitamins of the water-soluble type are also present, including B<sub>1</sub>, B<sub>2</sub> and C.

The enzymes present in milk include peroxidase, reductase, protease and lipase.

In the early stages of lactation the secretion (milk) is somewhat different from that produced when the function is well established. This form, known as *colostrum*, is especially characterised by the amount and condition of the proteins. Colostrum is, however, almost entirely of physiological interest and does not need specific description here.

In considering the many published analyses of milk, more especially of undomesticated animals, it must be remembered that the milk, as taken from the teat, varies in composition. In the case of a cow the first drawn milk may often contain less than 1% of fat, whilst the last drawn milk contains as much as ten times the fat found in the first drawn.

The milk of individual mammals of the same species also varies, as does also the milk from different breeds or varieties within the species.

Even the milk from individual teats of the same animal (the cow) has been shown to vary appreciably.

Some idea of the comparative gross variations in the milk of different mammals may be obtained from the following table.

#### AVERAGE COMPOSITION OF MILKS OF VARIOUS MAMMALS

Taken from "Fundamentals of Dairy Science," Associates of Rogers, American Chemical Society, Monograph Series No. 41, page 17

Species	Water, %	Casein, %	Albumin, %	Fat, %	Lactose, %	Ash, %
Human.....	87.41	0.91	1.23	3.76	6.29	0.31
Cow.....	87.27	2.95	0.52	3.66	4.91	0.69
Goat.....	84.14	3.04	0.09	6.00	5.02	0.81
Sheep.....	81.90	4.57	1.26	6.52	4.82	0.93
Buffalo.....	82.14	4.29	0.49	7.44	4.81	0.83
Camel.....	87.04	3.49	0.40	2.76	5.57	0.74
Horse.....	90.68	1.27	0.75	1.17	5.77	0.36
Ass.....	89.88	0.73	1.31	1.50	6.09	0.49
Reindeer.....	68.2	8.4	2.0	17.1	2.08	1.5



It will be noted that as regards the proportion of proteins and lactose the milks of the mare and ass agree closely with human milk.

*Composition of Cows' Milk.*—For the general purposes of analysis it is sufficient to distinguish two groups of solids, the fat and solids-not-fat, the latter being, of course, obtained by subtracting the fat from the total solids.

The grading of cows' milk from a bacteriological standpoint has tended to bring in to prominence the variability of milk. The same is true of the chemical composition. In the Sale of Milk Regulations 1901, an endeavour has been made to fix the presumptive minimum standards of 3% of fat and 8.5% of other solids, but while these figures are substantially lower than the average percentages of genuine milk, a proportion of samples of genuine milk fall below these limits.

From the point of view of the reasonable expectations of the consumer extensive series of analyses by Richmond indicate the average composition of milk as:

Fat.....	3.74%
Solids-not-fat.....	8.92%
Specific gravity.....	1.032

The milk of individual cows shows the greatest variation; Tocher found that, of 676 samples of the milk of individual cows, taken from different parts of Scotland, 8.3% contained less than 3% of fat, and 24.7% contained less than 8.5% solids-not-fat.

Cranfield, from the analyses of 730 samples of mixed milk of herds conducted at the Midland Agricultural College, found that 8.1% contained less than 3% of fat, and from the analyses of 518 samples from 10 herds, 11.6% contained less than 8.5% solids-not-fat.

### Circumstances Affecting the Composition of Milk

*Individuality of the Cow.*—This is a source of considerable variation, and as the milk yield can be improved by selection, so also can the percentage of fat and solids-not-fat.

*Intervals between Successive Milkings.*—The percentage of fat in the nights' milk after a short day interval is higher than the mornings' milk. This difference may exceed 1% of fat where the times of

milking are 6 a.m. and 3.30 p.m. This is the most frequent cause of low fat in the mornings' milk of herds milked at irregular intervals. It is not proved that the interval of time is the cause of this phenomenon. The percentages of solids-not-fat do not vary to any marked extent from this cause.

*Age of Cow.*—The butter fat tends to fall as the cow increases in age over 8 years. There is also a slight fall in the solids-not-fat in the milk of older cows.

*Breed.*—The percentage of fat differs in the milk of cows of different breeds. Tocher, from the analyses of milk of 676 random samples of cows' milk, gives the following average percentages of fat.

#### AVERAGES FROM LARGE NUMBERS OF EACH BREED

	%
Jersey.....	5.43
Guernsey.....	5.16
Kerry.....	4.67
Welsh.....	4.40
Ayrshire.....	4.09
Shorthorns.....	3.91
British Friesians.....	3.63

*Period of Lactation.*—The percentages of fat and solids-not-fat tend to fall during the first three or four months of lactation, from which time a steady increase is, as a rule, observed.

*Influence of Food.*—If cows are adequately and suitably fed, changes in food will not produce any permanent alteration in the percentages of fat and solids-not-fat. Even with bad or inadequate feeding, where the quantity of milk may be considerably altered, the percentages of fat and solids are not greatly affected.

*Abnormal Conditions.*—Excitement, particularly sexual excitement, worry and change of milkers, especially change to inexperienced milkers, will cause a considerable fall in the percentage of fat.

### Designations

Milk is now sold in England under designations under the Milk and Dairies (Amendment) Act 1922. The designations are as follows:

Designation	Herds		Max. No. of bacilli per c.c.	Coli-form bacillus	Other conditions
	Tuberculin tested at regular intervals	Veterinary inspected at various intervals			
Certified.....	Yes	Yes	30,000	- 1/10 c.c.	Bottled on farm, the name of farm, day of production and word "Certified" on each bottle cap.
Grade "A" tuberculin tested.	Yes	Yes	200,000	- 1/100 c.c.	Delivered to consumers in (a) the bottles or the sealed containers received from the farm; (b) suitable containers of not less than two gallons capacity; (c) bottles with the name of the dealer by whom the milk was bottled, the address of the licensed bottling establishment, the day of production and the words "Grade 'A' T.T." or "Grade 'A'" on each bottle cap.
Grade "A".....	No	Yes	200,000	- 1/100 c.c.	.....

### Pasteurised Milk

*Grade "A" Pasteurised.*—Grade "A" milk is that which after pasteurisation, as required by the Minister of Health, contains not more than 30,000 bacilli per cubic centimetre and no coliform bacillus in 1/10 c.c. All other conditions as required for Grade "A" milk.

*Pasteurised.*—Any milk that after pasteurisation, as required by the Minister of Health, contains not more than 100,000 bacilli per cubic centimetre. No requirement for bottling.

Although these designations refer to the bacteriological condition of the milk, it is well to remember that the certified milk bottled on the farm represents the milk of only a small number of cows, and so is subject to variation in chemical composition due to this cause.

### Sterilisation and Pasteurisation

Most of the milk now sold in cities has been subjected to the action of heat.

Under the Milk and Dairies (Amendment) Act, 1922, milk designated as "Pasteurised Milk" may be licensed under the following, among other, conditions:

1. The milk must be retained at a temperature of not less than 145° and not more than 150° F. for at least 30 minutes, and must then be immediately cooled to a temperature of not more than 55° F.
2. The milk must not be otherwise treated by heat and must not be pasteurised more than once.
3. Every vessel used for distributing the milk must be suitably labelled with the day of pasteurisation and the designation "Pasteurised Milk."

At this temperature, under 150° F., the destruction of the enzymes, precipitation of albumin, and the destruction of the cream line and other changes are not marked, as is the case with the old "flash" pasteurisation process, in which the milk was heated to 167° F. Undesignated pasteurised milk may have been twice heated and is a less reliable product.

Sterilised milk is heated in bottles to boiling point or above and for varying lengths of time and closed to exclude air while the bottles are still full of steam. In this milk greater chemical changes have taken place: peroxidases and other enzymes are destroyed, soluble proteins are rendered insoluble, and some of the calcium salts are rendered insoluble, thus preventing the rennin action. Vitamin B, and C are also partly destroyed.

**"Artificial Milk."**—It is now possible so to re-emulsify milk fat by means of emulsifiers that a product closely resembling milk can be obtained.

"Artificial cream" ("Artificial Cream Act 1929, Chap. 32, 19 & 20 Geo. 5") is also manufactured by this process.

The product, at its best, is made from very soluble consignments of spray-dried separated milk and unsalted butter. If these are weighed out in the right proportions, the slight difference in taste of the resulting product from fresh milk is the most definite means of distinguishing between the two. Detailed examination of the fat (see Butter) would disclose the use of fats other than butter fat. A reduction in the peroxidase test would distinguish the product from unheated milk. A test for nitrates might disclose tap water used to dissolve the milk solids (cf. Richmond, *Analyst.*, 1893, 18, 272; Lerrigo, *Id.*, 1930, 55, 433).

Satisfactory methods for certain identification by chemical or physical means of this new product have yet to be discovered.

The Milk and Dairies (Amendment) Act, 1922, 4 (1) prohibits the addition of dried or condensed milk, or of any fluid reconstituted therefrom, or of any skimmed or separated milk, to milk intended for sale.

### **Interpretation of Analytical Results**

The Sale of Milk Regulations, 1901, provide that a sample of milk that contains less than 3% of fat or less than 8.5% of other solids is to be presumed, for the purposes of the Food and Drugs (Adulteration) Act, 1928, not to be genuine until the contrary is proved.

Although these limits are substantially lower than the average, and although such milk does not come up to the reasonable expectation of the consumer, the fact that milk of individual cows and even of herds of cows not infrequently falls below these figures, does not justify a statement that the milk contains added water.

### **Added Water**

The addition of water is the most frequent adulteration to which milk is liable.

If the sample of milk is fresh the determination of the depression of freezing point will give reliable indication of watering, even if the solids-not-fat are above 8.5%. If the freezing point rises to  $-0.53^{\circ}$  C., watering may be suspected, and if to  $-0.52^{\circ}$  C., the milk has certainly been adulterated with approximately 5% of added water.

### **Analytical Processes**

The great importance of milk and milk products as foods, and their great liability to adulteration have led to extensive laboratory studies and to the development of many analytical processes. These are of two types—those especially adapted to ordinary milk inspection and control, and those adapted to research or to the verification of the ordinary methods.

The methods for ordinary inspection and sanitary control are principally the determination of fat and total solids. Specific gravity and ash are also often ascertained, and special points, such as detection of thickeners, colours, and preservatives, though now illegal, still require supervision. Methods for the detection of con-

tamination by dangerous animal products, such as pus cells, though of great practical importance, do not come within the scope of this article.

### Specific Gravity

Air-bubbles are held rather tenaciously by milk, and care must be taken in mixing to avoid as far as possible the inclosure of the air, and to allow sufficient time for the escape of any bubbles that may be present. The specific gravity of milk is understood to be taken at  $15.5^{\circ}$ ; samples should be brought near to this. The specific gravity of normal milk ranges between 1.028 and 1.035. The figure alone does not indicate the character of the sample, but taken in conjunction with the figure for fat or for total solids, it is of value as a check on the results furnished by other determinations.

The specific gravity rises gradually for some time after milk has been drawn, and the highest observation is to be made only after this action has ceased. This will require about 5 hours after the milk is drawn, if it has been kept below  $15^{\circ}$ , but at a higher temperature it will be necessary to allow at least 12 hours, although this rise is small. For all other data, the analysis should be made as soon as possible.

The specific gravity is usually taken with the lactometer, a delicate and accurately graduated hydrometer. The instrument must be immersed carefully so as not to wet the stem above the point at which it will rest. Its accuracy should be verified by immersion in distilled water at  $15.5^{\circ}$  and in milks of known specific gravity.

### Total Solids

The exact determination of the total solids in milk is complicated by the formation of a skin over the surface of the liquid, which hinders the free escape of moisture on further heating.

Various methods have been proposed to get over this difficulty; for example, the addition of 15 to 20 grm. of clean dry sand has been made; also, the milk has been coagulated with one drop of acetic acid, after which no skin forms. These methods are unnecessary, however, if a sufficiently small quantity of milk is taken in a flat-bottomed dish. The dish may be of any material which does not change weight on heating in air to the temperature of boiling water. Platinum, aluminium, nickel, silica or porcelain may be used.

A clean, dry, nickel dish, 9 cm. in diameter, with walls at least 1 cm. high, is weighed. Three to 5 grm. of the milk are placed in the dish and rapidly weighed. The milk is then distributed over the bottom by inclining the dish.

The dish with its contents is thereafter heated for about half-an-hour on a water bath and then dried in a water oven at  $100^{\circ}$  for two hours, after which it is cooled in a desiccator and weighed. It is then dried again in the oven for periods of half-an-hour till it ceases to lose weight.

### Ash

The determination of the ash in milk is a delicate matter, as milk contains chlorides which are volatile at high temperatures. The lowest possible temperature should therefore be used and the milk should never reach a dull red heat, ( $500^{\circ}$ ). A white ash should be obtained. The addition of strong nitric acid to the milk has been suggested, but is not recommended.

A flat-bottomed platinum dish is desirable but silica dishes may be used. Twenty to 25 grm. of milk are taken for ash. The milk is evaporated and afterwards ashed, with the precautions mentioned above; an electric muffle, with pyrometer attached, is convenient.

### Chlorides

Ten c.c. of milk are pipetted into a flask, 10 c.c. of  $N/20$  silver nitrate are then added, and the mixture shaken, and treated with 2 c.c. of 10% potassium permanganate solution and 10 c.c. of concentrated nitric acid (free from chlorine). The flask is then heated on gauze over a Bunsen flame until red-brown fumes appear. The liquid in the flask is now diluted to 150 c.c., and 2 c.c. of a saturated solution of iron alum in 10% nitric acid are added. The excess of silver nitrate is determined by titration with  $N/20$  potassium thiocyanate solution.

The result is calculated as mg. of chlorine ( $\text{Cl}_2$ ) per 100 c.c. of milk. This value varies from 70 to 170 and affords useful information when considered with the percentage of lactose (see p. 83). The osmotic pressure in natural milk is partly maintained by a rise in chlorine when the lactose falls.

### Phosphate and Calcium

The ash from 25 c.c. (weighed) of milk, as determined above, may be taken for the determination of phosphate and calcium.

The ash is dissolved in about 20 c.c. of 20% hydrochloric acid, and the solution neutralised with ammonia, the precipitate of calcium phosphate which forms being just redissolved in hydrochloric acid, and care being taken to avoid excess.

The calcium is then precipitated in the boiling fluid with 50 c.c. of a saturated solution of ammonium oxalate, the beaker left to stand overnight, and the reaction then adjusted with sodium acetate (20%) till faintly alkaline to methyl red.

The calcium oxalate is then filtered off and washed with 2% ammonia, followed by water, until the washings are free from oxalate.

The calcium oxalate precipitate is dissolved in 33% sulphuric acid, and the solution heated to 45 to 60°, and titrated with *N*/10 potassium permanganate solution. (1 c.c. = 0.002 Ca. or 0.0028 CaO.)

The filtrate and washings from the calcium oxalate are evaporated to dryness on a water bath, 5 c.c. of strong nitric acid added to the residue and again evaporated to dryness. This process is once more repeated. The residue is taken up in boiling distilled water and 140 c.c. of ammonium molybdate<sup>1</sup> added, and the beaker allowed to stand overnight, after which the liquid is filtered, and the precipitate washed with potassium nitrate (0.5%), until free from acid. It is then washed back into the original beaker with 20-30 c.c. of potassium nitrate solution, and titrated with *N*/2 sodium hydroxide, phenolphthalein being used as the indicator. (1 c.c. *N*/2 NaOH = 0.000674 grm. P<sub>2</sub>.)

### NITROGENOUS CONSTITUENTS

**Total Nitrogen** is estimated by the Kjeldahl method. The milk is weighed in a weighing bottle, and about 5 grm. transferred to a Kjeldahl flask, the exact amount being determined by reweighing the bottle.

<sup>1</sup> **Ammonium Molybdate.**—To 100 c.c. of water in large flask, add 50 grm. of molybdic acid and 100 c.c. of strong ammonia (sp. gr. 0.880). Shake till dissolved. Pour quickly, while stirring, into 720 c.c. of HNO<sub>3</sub> (sp. gr. 1.2). (This is made by making up 308.5 c.c. of strong acid to 720 c.c. with water.) Allow the mixture to stand overnight and filter.



Twenty c.c. of sulphuric acid, 10 grm. of potassium sulphate and 0.05 to 0.1 grm. of copper sulphate are added. When digestion is complete, and the liquid colourless, the flask is allowed to cool, and the neutralisation and distillation proceeded with as in the standard method. The percentage of nitrogen, multiplied by 6.38, gives an approximation of the total protein present in the milk.

**Casein (Moir's Method).**—Into a weighed covered beaker (100–150 c.c.) pipette 10 c.c. of milk and weigh again. Dilute with 50 c.c. of distilled water at 40–42°. Add 1.5 c.c. of 1.67 *N* (10%) acetic acid, and then stir gently by rotating the stirring rod four times in the beaker. After allowing the beaker to stand for about 20 minutes for the mixture to attain equilibrium, add 4.5 c.c. of 0.25 *N* sodium acetate and, after stirring gently, leave for at least an hour. Filter through a 9 c.m. No. 42 Whatman filter, folded in the fluted way.

Wash the precipitate with distilled water three times by decantation, follow by two further washings, and macerate the precipitate in the wash liquid and transfer it to the paper.

The filtration and washing should be carried out without interruption, and subsequently the casein adhering to the beaker and stirring rod should not be allowed to dry before being washed out with the sulphuric acid required for the Kjeldahl digestion. For this purpose about 20 c.c. of water are placed in the beaker and about 5 to 7 c.c. of the strong acid carefully poured down the side. The dissolved casein can be completely removed by three such treatments.

Place the filter paper and casein in the Kjeldahl flask into which the washings from the beaker have been poured; add the usual quantity of sodium or potassium sulphate and a trace of copper sulphate before commencing to heat the digestion flask.

Evaporate the water with a small flame to avoid frothing liable to occur at the end of the evaporation. Cool the flask just before digestion is complete, and wash back acids condensed on the neck of the flask with about 50 c.c. of water.

After again evaporating the water, dilute and distil with excess of caustic soda into standard acid, titrating back and expressing the result as percentage of casein nitrogen, or  $N \times 6.38$ , gives an approximation of the casein present. (*Analyst.*, 1931, 56, 147.)

**Albumin and Globulin.**—To the filtrate and washings from the casein precipitation add sufficient 20% trichloroacetic acid to make

the final concentration about 4%. Heat on the boiling water bath for at least half-an-hour. After cooling, filter and wash with a 1% solution of trichloroacetic acid. Rinse the beaker into the Kjeldahl flask in the same way as for casein: sometimes before adding any sulphuric acid a few c.c. of cold  $N/10$  NaOH may be used to remove any protein firmly attached to the beaker. The determination is completed as for casein and reported as percentage albumin plus globulin nitrogen.

**Casein and Globulin.**—Precipitate casein and globulin together by taking 10 c.c. of milk (weighed as for casein), neutralise, and mix with 90 c.c. of saturated sodium or magnesium sulphate solution. Add sufficient extra salt to saturate 10 c.c. of water. Filter off the protein precipitate, wash it with the saturated salt solution, and determine its nitrogen content by Kjeldahl's method.

The following scheme is suggested by Moir for the purpose of obtaining values for casein, albumin, and globulin in milk.

A. Casein by isoelectric precipitation at pH 4.6.

B. Casein and globulin by neutral saturated magnesium sulphate or sodium sulphate.

C. Total protein by warm 4% trichloroacetic acid.

From B — A globulin may be obtained; from C — B albumin may be obtained. (*Analyst.*, 1931, 56, 228.)

### Fat

Methods for the direct extraction of dried milk with ether present some difficulties, especially when the milk has been "homogenised" and the fat globules broken down. In such cases the last traces of fat are difficult to extract.

The Adams method, however, is still in use and gives satisfactory results with fresh milk. In this method 5 c.c. of milk are weighed in a tall weighing bottle which will take a roll of special fat-free paper. After weighing, the milk is soaked up by the paper, and the bottle quickly weighed again. The inverted coil is dried in the oven on a watch-glass and afterwards extracted in a Soxhlet extractor till free from fat. Special precautions are taken that the surfaces of the coil do not touch; this can be prevented by rolling a length of copper wire with the paper.

A blank extraction should be carried out with a similar coil of the paper and a lighter flask, and this flask is used as a tare after the

ether has been evaporated into the Soxhlet at the end of the extraction, and the last traces removed by drying in an oven at 100°. The flasks should be weighed at intervals of half an hour till constant in weight.

**Röse-Gottlieb Method** (A. O. A. C.).—Place 10 to 11 grm. in a Röhrig tube or similar apparatus, add 1.25 c.c. of strong ammonium hydroxide (2 c.c. if the sample is sour) and mix thoroughly, and then add 10 c.c. of 95% alcohol and mix again. Add 25 c.c. of washed ether, shake well for 30 seconds, add 25 c.c. of petroleum spirit (redistilled below 60°) and shake again for 30 seconds. Allow the vessel to stand until the upper layer is clear (about 20 minutes usually). Draw off as much as possible of the fat solution (usually about 0.5 to 0.8 c.c. will remain) into a weighed flask through a small rapidly-acting filter. The flask should be tared with a similar one as counterpoise. Again extract the material with 15 c.c. of each solvent, shaking well for 30 seconds after each addition, and pass the solution through the same filter. Wash the spigot of the apparatus, the tip of the funnel and the filter with a few c.c. of a mixture of the solvents in equal parts, free from suspended water. A third extraction, carried out as before, is necessary, but usually yields less than 1 mg. if the extraction has been carried out well. Evaporate the solution of fat slowly on a steam bath, and dry in a boiling-water oven to constant weight. Test the purity of the fat by solution in a little petroleum spirit. If a residue is left, remove the fat completely with petroleum spirit, dry the residue, weigh and deduct this weight. A blank test should be made of the reagents used.

**Centrifugal Methods.**—Although almost all the fat of milk may be separated by the centrifuge, the emulsion is not destroyed, and the volume of cream is merely suggestive as to the fat-content of the milk. To obtain a clear fatty layer in condition for close measurement, it is necessary to use chemicals. The methods at present most employed depend essentially on one devised by Gustaf DeLaval, who, on July 1, 1885, took out a patent in Sweden for the use of a mixture of 20 volumes of strong acetic acid and 1 volume of strong sulphuric acid. This mixture coagulates and then dissolves the proteins, destroys the emulsion, but does not otherwise affect the fat and does not act on the lactose. By brief whirling in a centrifuge the fat collects in a clear sharply defined layer. DeLaval took out patents in several countries subsequent to the above date.

In 1889, **Leffmann and Beam** devised a method in which a small amount of amyl alcohol with an equal volume of hydrochloric acid was added to the milk, and the proteins thus coagulated dissolved by strong sulphuric acid. About the same time **Babcock** devised a process in which sulphuric acid was used alone. Subsequently **Gerber** published a process in which the use of amyl alcohol and sulphuric acid was advised.

**Leffmann-Beam Method.**—The test-bottles have a capacity of about 30 c.c. and are provided with a graduated neck, each division of which represents 0.1% by weight of butter fat.

Fifteen c.c. of the milk are measured into the bottle, 3 c.c. of a mixture of equal parts of amyl alcohol and strong hydrochloric acid added, mixed, the bottle filled nearly to the neck with concentrated sulphuric acid, and the liquids mixed by holding the bottle by the neck and giving it a gyratory motion. The neck is now filled to about the zero point with a mixture of sulphuric acid and water prepared at the time. It is then placed in the centrifugal machine, which is so arranged that when at rest the bottles are in a vertical position. If only one test is to be made, the equilibrium of the machine is maintained by means of a test-bottle, or bottles, filled with a mixture of equal parts of sulphuric acid and water. After rotation for from 1 to 2 minutes, the fat will collect in the neck of the bottle, and the percentage may be read off. It is convenient to use a pair of dividers in making the reading. The legs of these are placed at the upper and lower limits, respectively, of the fat, allowance being made for the meniscus; one leg is then placed at the zero point and the reading made with the other. Experience by analysts in various parts of the world has shown that with properly graduated bottles the results are reliable. As a rule, they do not differ more than 0.1% from those obtained by the Adams process, and are generally even closer.

The mixture of fusel oil and hydrochloric acid seems to become less satisfactory when long kept. It should be clear and not very dark in colour. It is best kept in a bottle provided with a pipette which can be filled to the mark by dipping. Rigid accuracy in the measurement is not needed.

**Babcock Method.**—By means of a pipette which delivers 17.6 c.c., (the approximate volume of 18 grm. milk), bottles, suitably graduated, are charged with this volume of the sample and 17.5 c.c. of

sulphuric acid (sp. gr. 1.820–1.830), added by portions until the coagulum first formed is dissolved. The bottle is shaken until all traces of curd have disappeared. Counter-balanced bottles are then whirled for 5 minutes in a centrifuge at speeds varying with the diameter of the wheel, from 1,000 revolutions with an 11" wheel to 700 revolutions with a 24" wheel. Hot (60°), distilled water is added to fill the bottle to the beginning of the stem, and the bottle is whirled for two minutes. Enough hot water is then added to bring all the fat into the graduated part of the neck, when, after one minute's whirl, the bottles are removed to the hot water bath preparatory to reading. The bottles should be immersed in the water bath to the level of the top of the fat column; the fat should be clear and of a golden yellow colour. The bottles are read in the same way as the Leffmann-Beam bottles, which they resemble in shape.

**The Gerber Method.**—The bottles or butyrometers for the Gerber method are of a different shape; they are inverted for the purpose of filling and are afterwards firmly corked with a rubber stopper before the liquids used in the test are mixed.

The centrifuge consists of a slightly inclined disc, fitted with brass containers for the butyrometers, and closed with a lid. It may be whirled by hand or by power (electric motor), but in either case the speed should be 1,000 revolutions per minute.

To the inverted butyrometers are added in the following order, 10 c.c. of sulphuric acid (sp. gr. 1.820–1.825), 1 c.c. of amyl alcohol (sp. gr. 0.8145 to 0.816), and exactly 11 c.c. of milk from a standard pipette. The acid and amyl alcohol are conveniently delivered from automatic measures. The butyrometers are then firmly corked with dry rubber stoppers, rapidly and thoroughly shaken, till all the liquids are mixed and the curd dissolved. They are then placed in the centrifuge with the graduated necks pointing to the centre and spun for four to five minutes at 1,000 revolutions per minute, after which they are taken out and placed in a vertical position, fat uppermost, in a water-bath at 150°–155° F. On removing them from the water-bath for reading, the rubber stopper is eased out till the bottom of the fat layer coincides with a long line indicating a whole number; the full percentages of fat are then counted, and the small divisions, indicating 0.1%, added. In reading, the position of the bottom of the meniscus at the top of the

fat column, when level with the eye, is taken, and it is possible to estimate the second place of decimals. With practice, readings of different estimations should not differ by more than 0.05% of fat. Any test which fails to give a clear layer of fat should be discarded, though this should not occur. Gerber butyrometers with stems oval in cross section are preferred.

The Babcock test is much used in colonial creameries and in America. The Gerber test is favoured in England.

Many countries provide that all glassware used for estimating fat should be tested and marked with a Government stamp. In England the National Physical Laboratory carries out this service and provides a leaflet giving specifications, etc.

With such calibrated glassware objections to mechanical milk tests are largely removed, but the results should be checked by gravimetric methods from time to time and always for legal cases.

## LACTOSE

For gravimetric estimation, the A. O. A. C. employs Soxhlet's method with the following reagents:

*Copper Sulphate Solution.*—34.639 grm. of pure crystallised copper sulphate are dissolved in water and made up to 500 c.c.

*Alkaline Tartrate Solution.*—17.3 grm. of pure sodium potassium tartrate and 50 grm. of good sodium hydroxide are dissolved in water, and the solution made up to 500 c.c.  $N/2$  sodium hydroxide.

*Determination.*—25 c.c. of the sample are placed in a 500 c.c. graduated flask and diluted with 400 c.c. of water and 10 c.c. of the copper sulphate solution and 8.8 c.c. of  $N/2$  sodium hydroxide solution added. The mixture should still have an acid reaction and contain copper in solution. If this is not the case, the experiment must be repeated, using a little less of the alkali. The flask is filled to the mark with water, shaken, and the liquid passed through a dry filter. 50 c.c. of Fehling's solution, obtained by mixing equal parts of the copper sulphate and alkaline tartrate solutions, are heated to brisk boiling in a 300 c.c. beaker, 100 c.c. of the filtrate, obtained as above, are added, and boiling continued for 6 minutes; the liquid is then promptly filtered through asbestos, and the copper determined by one of the methods described in detail in Vol. I, pages 456 *et seq.*

**Polarimetric Determination** (A. O. A. C.).—Prepare an acid mercuric nitrate solution by dissolving mercury in twice its weight of

strong nitric acid (sp. gr. 1.42) and diluting with an equal volume of water. Determine the specific gravity of the milk. The volume of the sample to be taken depends on the specific gravity, and is to be measured at the same temperature at which that datum is obtained. This volume is to be determined by the annexed table.

Put the quantity of milk indicated in a flask graduated at 102.6 c.c. Add 1 c.c. of the acid mercuric nitrate solution (a slight excess will do no harm), fill to the mark with water, shake, pass through a dry filter and polarise. It is not necessary to heat. The reading is intended to be obtained on a 100 mm. tube; if longer tubes are used, the reading must, of course, be divided proportionately.

VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT  
(Ventzke scale)

Sp. gr.	Volume c.c.	Sp. gr.	Volume c.c.
1.024	64.25	1.031	63.80
1.025	64.20	1.032	63.75
1.026	64.15	1.033	63.70
1.027	64.05	1.034	63.65
1.028	64.00	1.035	63.55
1.029	63.95	1.036	63.50
1.030	63.90		

### Acidity

The titratable acidity of milk is determined in a flat white porcelain dish, 10 c.c. of milk being taken for the test and about 10 c.c. in a similar dish for comparison of colour. Six or seven drops of 0.5% solution of phenolphthalein in alcohol are added to the first dish only, while to the control dish about 1.4 c.c. of *N*/9 sodium hydroxide are added for fresh milk and more for acid samples.

*N*/9 sodium hydroxide, free from carbonate, is carefully added from a standard burette, with constant stirring, to the first dish, the additions being made drop by drop as neutralisation is approached. The end-point is taken when the first tinge of pink can be observed on comparing the two dishes.

The number of c.c. required, divided by 10, will give the equivalent as lactic acid.

The titratable acidity of fresh milk from different sources may vary from 0.14 to 0.2, although no lactic acid is present.

The test is of importance in cheese making and in the determination of the freezing point of milk, for which no increase in acidity should have taken place.

### Citrates

**Desmoulière's Method** (*Bull. Scien. Pharm.*, 1910, **17**, 588).—Two hundred c.c. of the milk are mixed with 100 c.c. of 2% acetic acid and boiled for a short time under a reflux condenser. The liquid is cooled, filtered through a dry filter, 150 c.c. of the filtrate mixed with 3 grm. of diatomaceous earth, or similar inert powder, and evaporated on the water-bath to a pasty condition. The residue is cooled, 3 c.c. of dilute (1:5) sulphuric acid added and the mixture allowed to stand for several hours with occasional stirring. An additional portion of 3 grm. of the inert powder is then stirred in, and the mass repeatedly extracted with cold ether saturated with water. The extracts, which may measure as much as 1,000 c.c. in all, are mixed and distilled to small bulk at as low a temperature as possible. The residue is treated with water, made up to a known volume, and divided into three equal portions. In one portion the total acidity is determined, in the second the volatile acid (acetic), and in the third the phosphoric acid. The last named may not be present, but the test must always be made for it. The citric acid is found by taking the difference between the total acidity and the sum of the acidities due to the volatile acids and the phosphoric acid. In examining human milk, the amount of sulphuric acid may be advantageously reduced to even 2 c.c., but with cow's milk the full amount given above should be used.

### General Calculation Methods

In 1879, Behrend and Morgen (*J. Landw.*, 1879, **27**, 250) pointed out a relation between the sp. gr., fat and total solids of milk and published a table of calculations. Clausnitzer and Mayer (*Forschung. auf Gebiete Agric.*, 1879, **2**, 265) reported further on the method. Hehner (*Analyst*, 1883, **8**, 129) compiled a formula based on analyses by Wanklyn's method. He took the sp. gr. of fat as 0.9278, and assumed that 1% of this decreased the sp. gr. of milk by 0.725 (water being 1,000), whilst each unit of solids-not-fat increased the sp. gr. by 3.6. Fleischmann and Morgen (*J. Landw.*, 1882, **30**, 293) constructed a formula from data obtained by extract-



ing fat from the residue after drying the milk absorbed by calcium sulphate. They assumed the sp. gr. of milk-fat as 0.940 at 15°, but Fleischmann subsequently found 0.930 to be more nearly correct and altered the formula accordingly. Hehner and Richmond (*Analyst.*, 1888, 13, 523) established a formula based on fat extraction by the Adams method. Later, Richmond (*Analyst.*, 1889, 14, 121) revised this and gave the following,

$$t = 0.25g + 1.2f + 0.14$$

in which *t* is total solids; *f*, fat; *g*, the last two integers of the sp. gr. and any decimals (water being 1,000). Fleischmann's formula, as given in German works, is

$$t = 1.2f + 2.665 \left( \frac{100g - 100}{100} \right)$$

the letters having the same significance. Babcock's formula has been much used in the United States. It is adapted to calculating the solids-not-fat. In this formula *g* is the entire figure for sp. gr., referred to water as 1.

$$\text{S. N. F.} = \left( \frac{100g - fg}{100 - 1.0753fg} - 1 \right) \times 2.5(100 - f)$$

Babcock has also given a much simpler form adapted for total solids; this differs but slightly from Richmond's formula. A detailed investigation of the relations between the gravimetric results and the figures obtained with these formulae has been published by Shaw and Eckles (*Bull.* 134, *Bur. An. Indust.*, U. S. Dept. Agric.).

**Adulterations.**—By far the larger part of the laboratory work on milk is for assistance in the sanitary control of the supply, and the analyses are principally directed to the detection of the ordinary forms of adulterations. The most important of these are: skimming, and watering. Skimming and watering are suspected from results obtained by the determination of fat and total solids; from these data the solids not fat are calculated. For the ordinary purposes of milk control, fat can be determined with quite sufficient accuracy by centrifugal methods. The total solids may be determined directly as described on p. 75.

Judgment whether a given sample has been skimmed or watered depends in many cases upon the standard for whole milk (*i. e.*, milk

in the condition in which it is drawn from the animal). Great irregularity of standards for fat and solids-not-fat exists, and the opinion of the analyst will be determined, therefore, by the standard of the locality. In most cases the standard for fat is between 3 and 4%, and that for solids-not-fat about 8.50%. (See p. 70.)

As fat diminishes the sp. gr. of milk, and the other solids increase it, it is possible to take off a small amount of the former and add some water without disturbing the sp. gr., but, of course, the above analytical methods will detect this procedure. It is now admitted that, except in cases of wide departure from the usual limits, the adulteration of milk cannot be detected by the sp. gr. alone, but the employment of a carefully graduated lactometer is of use in routine milk inspection.

### Direct Detection of Added Water

*The Freezing-Point Method.*—As mentioned in the opening paragraphs of this article, the freezing point of genuine fresh milk more nearly approaches a physical constant than that of any other determination which can conveniently be made. The exact determination of the true freezing point to the third place of decimals involves many corrections, but an approximate figure, agreeing in repeat determinations to  $\pm 0.005^\circ$ , can be made without much difficulty.

The simple form of Beckmann's freezing-point apparatus may be used, as recommended by R. L. Andrew (*Analyst*, 1929, 54, 210).

A convenient modification is the replacement of the Beckmann thermometer by a standard solid-stem thermometer similar to that recommended for use with the Hortvet cryoscope, (*J. Ind. Eng. Chem.*, 1921, 13, 198; *J. Assoc. Off. Agr. Chem.*, 1922, 5, 172, 470, 484).

The use of the Hortvet cryoscope is an alternative method recommended by the A. O. A. C.

The method described by Andrew consists in the use of two freezing mixtures of ice and salt. The Beckmann apparatus is filled with a weak freezing mixture ( $-2$  to  $-4^\circ$ ). A jar containing a very strong freezing mixture is also prepared.

The freezing point of recently boiled and cooled distilled water should be determined at the start and finish of each series of determinations, with the object of checking the zero point of the thermometer.

For this purpose, the inner vessel is filled with sufficient water to cover the bulb. The tube containing the distilled water is then placed in the strong freezing mixture, so that the level of the water in the tube is just above the level of the freezing mixture. The water is stirred slowly until a skin of ice is formed on the inside of the tube, when slight super-cooling usually occurs.

The tube is then removed from the strong freezing mixture, wiped dry with a cloth, and warmed slightly with the hand, so that the ice can be detached from the walls of the tube and broken up with the stirrer.

The tube is then placed in the Beckmann apparatus and stirring continued till the mercury column remains stationary for some time. The thermometer should then be read carefully, two or three times, preferably with a reading telescope, avoiding errors of parallax. The average of this reading and of a similar observation taken at the end of the series, when both have been corrected for scale errors of the standard thermometer, is taken as the freezing point of water.

The emptied and dried inner tube is next filled with milk to the same level as used in the case of the water, and the process of stirring repeated, with the tube similarly immersed in the strong freezing mixture. The mercury falls rapidly while the milk is stirred, until some solid phase begins to form, when it will begin to rise. At this stage, the tube is rapidly removed, dried and any solid detached from the sides of the tube. It is then placed in the Beckmann apparatus and stirred till the rising column comes to rest. When steady, several readings of the mercury column are taken.

The algebraic difference between this reading, corrected from the certificate of the standard thermometer used and the freezing point of water, gives the freezing point of the milk.

The freezing point with genuine fresh milk will usually be about  $-0.55^{\circ}$ . If it is nearer to zero than  $-0.53^{\circ}$ , watering may be suspected, and if it is  $-0.52^{\circ}$ , 5% of water has been added. The percentage of added water may be calculated with more certainty from this determination than from any other convenient estimation.

The samples of milk must be fresh for this determination and the titratable acidity should not exceed that commonly found in milk of the locality.

**Zeiss Immersion Refractometer Reading.**—The use of the Zeiss immersion refractometer for ascertaining the refractive index of the

wey (milk-serum) offers a rapid and satisfactory method for detecting watering. Several methods of preparing this wey have been proposed, but Lythgoe (*Rep. S.B. of Health of Mass.*, 1909) has found, as the result of extended experience, the following to be the best yet suggested.

Dissolve 72.5 grm. of crystallised copper sulphate in water and dilute to 1,000 c.c. If this solution does not give a refraction of 36 on the scale of the immersion refractometer at 20°, add water or copper sulphate until the desired result is obtained. To 8 c.c. of the copper solution add 32 c.c. of milk. Shake well and pour upon a dry filter. When the filtrate begins to come through clear, change the receiver, pour the small quantity of cloudy filtrate upon the filter, and continue the filtration as usual. Take the refraction of the clear filtrate at 20°, by means of the immersion refractometer. A reading below 36 indicates added water.

Another method of preparing milk for ascertaining the refractive index is as follows:

*Acetic Acid Serum Method.*—To 100 c.c. of milk at 20° in a beaker add 2 c.c. of 25% acetic acid (sp. gr. 1.035). Cover and place in a bath at 70° for 20 minutes, then in ice-water for 10 minutes. Separate curd by rapid filtration through a small filter. Take the reading by the Zeiss immersion refractometer when in the constant temperature bath at 20°, to be determined by a thermometer graduated in tenths of a degree. A reading below 39 indicates added water; with a reading between 39 and 40 the addition of water is to be suspected. If the reading is 40 or below, proceed as directed in the next paragraph.

Transfer 25 c.c. of the serum to a weighed flat platinum basin, evaporate to dryness on a water bath. Heat with a low flame (to avoid spirting) until the mass is thoroughly charred, ignite the mass in an electric muffle oven, preferably with pyrometer control, to a white ash at a temperature not above 500°. Express the result in grm. per 100 c.c. A result below 0.715 indicates added water. (A. O. A. C. method, with some changes in phraseology.)

### Detection of Heated Milk

Fresh milk contains one or more enzymes of the "peroxydase" type, that is, having power to bring about transfer of oxygen

from peroxides to oxidisable substances. As the function of these enzymes is destroyed at temperatures near  $100^{\circ}$ , it becomes possible to utilise the reaction for determining whether a given sample has been thus heated. In most cases the action of the enzyme is indicated by the production of a colour, no change occurring when the enzyme has been heated. Hydrogen peroxide is commonly employed for furnishing the oxygen. A considerable number of substances have been found to be susceptible to oxidation under the influence of the milk enzymes. Benzene derivatives commonly used as photographic developers are especially susceptible. Guaiacum was first used, however. This was employed by Arnold (*Jahresber. d. Königl. Tierärztl. Hochsch.*, 1880-82, 161).

**Arnold's Method.**—According to Arnold and Menzel (*Milch Zeit.*, 1902, 247), solution of guaiacum in acetone is better than the ordinary tincture. The test is applied by adding to a small amount of the sample in a test tube, about 10 drops of the guaiacum solution, to which a drop or two of hydrogen peroxide solution has just been added, so that the reagent will float on the milk. If the sample has not been heated above  $80^{\circ}$ , the point of contact of the liquids will show a deep blue ring.

*Ortol* and hydrogen peroxide give a red colour in unheated milk.

**Para-phenylenediamine Test.**—One part of the sample of milk to be tested is made up to 5 parts with boiled milk. A measured quantity of this diluted milk is made up to 10 c.c. with boiled milk, and 0.3 c.c. of a 1% solution of hydrogen peroxide and 0.25 c.c. of a 2% solution of para-phenylenediamine are added. The blue colour obtained at different dilutions of the sample of milk affords a measure of the amount of peroxylase remaining in the sample. Comparisons should be made with genuine diluted fresh milk half an hour after the reagents have been added. Zilva (*Biochem. J.*, 8, 650), points out that the inactivation is gradual; the velocity of the reaction of the enzyme becomes marked at about  $67^{\circ}$ , and at  $73^{\circ}$  the inactivation proceeds so fast that it may be considered almost "instantaneous." At  $71^{\circ}$  96% of the total peroxylase is destroyed in 20 minutes.

**Schardinger Reaction.**—This depends on the anaerobic action of heated and unheated milk on methylene blue in the presence of formaldehyde at  $45^{\circ}$ . Heated milk remains blue, but from unheated milk the colour disappears. (See also Vol. VIII, 181, 185.)

## COLOURING MATTERS

Annatto, turmeric, and some coal-tar colours have been used for colouring milk. Caramel has been occasionally used, saffron and carotene but rarely. All these are now prohibited. Annatto may be detected by rendering the sample slightly alkaline with sodium bicarbonate, immersing a slip of filter paper in the liquid, and allowing it to remain overnight. Annatto will produce a reddish-yellow stain on the paper.

*Annatto.*—Leys gives the following method for detecting annatto: 50 c.c. of the sample are shaken with 40 c.c. of 95% alcohol, 50 c.c. of ether, 3 c.c. of water, and 1.5 c.c. of ammonium hydroxide solution (sp. gr. 0.900), and allowed to stand for 20 minutes. The lower layer, which in presence of annatto will be greenish-yellow, is drawn off and gradually treated with half its volume of a 10% solution of sodium sulphate, the separator being inverted without shaking, after each addition. When the casein separates in flakes which gather at the surface, the liquid is drawn off, strained through wire gauze, and placed in four test-tubes. To each of these amyl alcohol is added, and the tubes are shaken and immersed in cold water, which is gradually raised to 80°. The emulsion breaks up, and the alcohol, holding the annatto in solution, comes to the surface. The alcoholic layer is separated from the lower stratum, evaporated to dryness, and the residue dissolved in warm water containing a little alcohol and ammonium hydroxide. Clean white cotton is then introduced, and the liquid evaporated nearly to dryness on the water-bath. The cotton, which is coloured a pale yellow, even with pure milk, is washed and immersed in a solution of citric acid, when it will be immediately coloured rose-red if the milk contained annatto. Saffron, turmeric, and the colouring-matter of the marigold do not give a similar reaction.

*Coal-tar colours* may often be detected by dyeing wool, but Lythgoe has devised the following method, which is satisfactory: 15 c.c. of the sample are mixed in a porcelain basin with an equal volume of hydrochloric acid (sp. gr. 1.20), and the mass shaken gently so as to break the curd into coarse lumps. If the milk contains an azo-colour, the curd will be pink; with normal milk the curd will be white or yellowish.

**General Method for Colours in Milk.**—Leach has devised a general method. 150 c.c. of the sample are coagulated in a porcelain basin, with the addition of acetic acid and heating, and the curd separated from the whey. The curd will often collect in a mass; but if this does not occur, it must be freed from whey by straining through muslin. The curd is macerated with ether for several hours in a closed flask, with occasional shaking, to extract fat. Annatto will also be removed by it. The ether and curd are separated and treated as follows:

The ether is evaporated, the residue mixed with a little weak sodium hydroxide solution, and passed through a wet filter; and when this has drained, the fat is washed off and the paper dried. An orange tint shows annatto, which may be confirmed by a drop of stannous chloride solution, which makes a pink spot.

If the curd is colourless, no foreign colouring-matter is in it; if orange or brown, it should be shaken with strong hydrochloric acid in a test-tube.

If the mass turns blue gradually, caramel is probably present. The whey should be examined for caramel.

If the mass turns pink at once, an azo-colour is indicated.

## PRESERVATIVES

These are now illegal. Preparations of boric acid and of borax were at one time the most frequent; *formalin* (a 40% solution of formaldehyde) was also much used. Sodium carbonate is occasionally used to prevent coagulation due to slight souring. Fluorides and "abrostol" are possible preservatives. A mixture of boric acid and borax is more efficient than either alone.

The only permissible method of preserving milk is refrigeration.

In addition to the descriptions of the detection and estimation of preservatives given below, see also under "Cream."

**Formaldehyde.**—*Hehner's Test.*—Hehner found that when milk containing formaldehyde is mixed with sulphuric acid containing a trace of a ferric compound, a distinct blue appears (*Analyst*, 1896, 21, 94). Richmond and Boseley (*Analyst*, 1896, 21, 92) showed that the delicacy of the test is much increased if the milk is diluted with an equal volume of water, and sulphuric acid (90 to 94%) added so that it forms a layer underneath the milk. Under these conditions milk, in the absence of formaldehyde, gives a slight greenish tinge at

the junction of the two liquids, whilst a violet ring is formed when formaldehyde is present, even in so small a quantity as 1 part in 200,000 of milk. The tint is permanent for 2 or 3 days. In the absence of formaldehyde, a brown ring may form in the course of a few hours, but it is below the junction line of the two liquids.

**Phenylhydrazine Test.**—The following test avoids the fallacy of some other tests. A pinch of phenylhydrazine hydrochloride is added to a few c.c. of the sample, the liquid shaken, and a drop of a fresh solution of sodium nitroprusside and a few drops of sodium hydroxide solution added. A greenish tint is at once produced if formaldehyde is present.

**Benzoates and Salicylates.**—The following method (*Ann. chim. anal.*, 1909, **14**, 53) covers both these preservatives.

Ten c.c. of dilute sulphuric acid (5%) are added to 20 c.c. of 95% alcohol, and into this 50 c.c. of the milk are poured in a fine stream, with constant stirring. After a few moments, the mixture is filtered, the filtrate being returned until it passes clear. A sufficient volume of the filtrate is extracted in the usual manner with an equal volume of ether or similar solvent. The solvent is divided into two portions, which are separately evaporated and tested for benzoic and salicylic acids, respectively, as given below.

**Benzoic Acid.**—This is detected by a modification of Mohler's method by Von der Heide and Jakob (*Z. Nahr. Genussm.*, 1910, **19**, 137). The text is from *Cir. No. 136, Bur. Chem. U. S. Dept. Agric.*,

The residue that is to be tested for benzoic acid is dissolved in a little water, the solution mixed with from 1 to 3 c.c. of *N* sodium hydroxide solution, and evaporated to dryness. To this residue are added from 5 to 10 c.c. of concentrated sulphuric acid and a small crystal of potassium nitrate, and the mixture heated either for 10 minutes in a glycerin bath between 120° and 130°, or for 20 minutes in boiling water. If the glycerin bath is used, the temperature must not be permitted to go over 130°. Metadinitrobenzoic acid is formed. After cooling, 1 c.c. of water is added, the liquid made decidedly ammoniacal, boiled to break up ammonium nitrite, and some fresh colourless ammonium sulphide solution added in such a way that the liquids do not mix. A brown ring at the junction of the liquids indicates benzoic acid. The liquids being mixed, the colour diffuses and, on heating, changes to greenish-yellow. The last reaction distinguishes benzoic acid from salicylic and cinnamic



acid, as these form amino-derivatives which are not destroyed by heating. Phenolphthalein interferes with this process.

**Salicylic Acid.**—The other portion of the ether-extract may be evaporated and tested for salicylic acid in the usual manner with a ferric compound.

**Sodium Carbonate and Sodium Hydrogen Carbonate.**—These substances are occasionally added to milk to prevent acidity due to decomposition. Barthel recommends a test devised by Hilger: fifty c.c. of the milk are diluted with 250 c.c. of water, the mixture is heated, precipitated with a small amount of alcohol, and a convenient volume filtered. The filtrate is evaporated to half its bulk. The presence of an alkali carbonate is easily ascertained by the usual tests.

**Borates.**—Jenkins's method (*Rep. State Expt. Sta., Conn., 1901, 106*) is convenient and reasonably delicate. Ten c.c. of milk are mixed with 7 c.c. of hydrochloric acid, filtered, and a strip of turmeric paper dipped in the filtrate, and then dried on a watch-glass on the water-bath. The paper becomes red in the presence of borates.

A simple test is to mix in a porcelain basin a drop or two of the milk, a drop of hydrochloric acid, and a drop of alcoholic solution of turmeric and evaporate to dryness on the water-bath. The residue, touched with ammonium hydroxide, will show a distinct greenish stain in the presence of very small amounts of borates.

It is obvious that the delicacy of both these tests may be materially increased, so that they will suffice for all purposes, by concentrating the sample. As boric acid is volatile with steam, it is best to render the sample slightly alkaline with sodium hydroxide before evaporating.

## ORGANIC POLLUTIONS

Several chemical tests have been published by which it is claimed that an approximate estimation of contaminating organisms and substances can be made, but they are not capable of replacing the exact methods of the pathological and bacteriological laboratory.

**Reductase Test.**—To 40 c.c. of milk in a test tube, 1 c.c. of a standard solution of methylene blue is added.<sup>1</sup> The contents of

<sup>1</sup> A final dilution of 1 part of the dye in 270,000 of milk is used in Europe. In America, where 10 c.c. of milk are used for the test, tablets of the dye are dissolved in 800 c.c. of water, and 1 c.c. of this added to 10 c.c. of milk, giving a dilution of 1 in 200,000; or a stock solution is made by dissolving 1.1 grm. of the dry dye in 500 c.c. of water and 1 c.c. of the stock solution, diluted with 39 c.c. of water for use.

the tube are carefully mixed, and the tube placed in a water bath maintained at a temperature of  $38^{\circ}$  to  $40^{\circ}$ . The length of time which elapses before the blue colour disappears is noted. The best milk, of Certified standard quality, remains coloured for more than five and a half hours; for other milks the following grading is recommended, based on the reduction time or the length of time which elapses before the blue colour disappears. Class I (Good milk) five and a half hours. II (Fair) Two to five and a half hours. III (Bad milk) 20 minutes to 2 hours. IV (Very bad milk) 20 minutes or less. See also Vol. VIII, 188.

### **Preservation of Samples for Analysis**

For the preservation of milk samples for a day or two, refrigeration is the best method. Several preservatives have been proposed for keeping samples. Richmond found small amounts of hydrofluoric acid effective, but it has been but little used. Formaldehyde is very efficient; in large amount, however, it increases the total solids, interferes with the reactions of the proteins, and simulates some reactions of the carbohydrates. Two drops of commercial formalin to 25 c.c. will preserve a sample for several days.

Mercuric chloride may also be used, but in this case sample bottles should be labelled "poison." Half a c.c. of a 5 per cent solution of mercuric chloride in alcohol is added to an empty 200 c.c. sample bottle, and the alcohol allowed to evaporate. This method is used in New Zealand for samples in which the freezing point is to be determined.

Potassium dichromate is used for samples in which fat tests only are to be made, a sufficient quantity being used to turn the whole sample to a pale yellow colour.

### **MILKS OTHER THAN COWS' MILK**

The processes of analysis given in this article are, in general, applicable to all forms of milk, using the term in the sense in which it is given at the beginning of the article. It must, however, be borne in mind that much remains to be done in the isolating and identifying the ingredients in less-used milks, and that it is not unlikely that the protein and carbohydrate constituents are materially different in the milks of animals of widely different nature.

In analytical methods involving coagulation, especially with the object of enclosing the fat and thus obtaining a clear filtrate for examining the carbohydrates, it must be noted that with milks low in proteins, such as human milk, the coagulum may be too scanty to accomplish the purpose, and filtration through very close filters or clarification by the use of the centrifuge may be needed. It is possible that the addition of a small volume of fresh white of egg may aid the operation or the addition of a little china clay.

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# MILK PRODUCTS

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The products obtained by treating milk in various ways are only second in importance to milk itself. In the following section, the chemistry of the chief of these is considered, together with details of manufacture, etc., in so far as they are of interest to the analyst.

The following products are dealt with:

Section A.—(a) Cream; (b) Cream Substitutes; (c) Artificial Cream; (d) Tinned Cream; (e) Clotted Cream; (f) Skim Milk.

Section B.—(a) Butter Milk; (b) Whey; (c) Milk sugar; (d) By-products of the manufacture of Milk sugar.

Section C.—Condensed Milk.

Section D.—Specially-Treated Milks. (a) Humanised Milks; (b) Diabetic Milk; (c) Peptonised Milk; (d) Homogenised Milk; (e) Sterilised Milk; (f) Buddeised Milk; (g) Irradiated Milk.

Section E.—Sour and Fermented Milks. (a) Koumiss; (b) Kephyr; (c) Mazun; (d) Yoghout.

Section F.—Infants' Foods.

Section G.—Dried Milk.

Section H.—Cheese.

## SECTION A (a) CREAM

**CREAM** is the product which is separated from milk and consists of an artificial agglomeration of the fat corpuscles of the original milk, brought about either by gravitation or mechanical (centrifugal) power. The colour varies from pure white to a deep yellow, according to the breed of cattle and time of year, the usual tint being a very faint yellow.

Cream obtained by gravitation is now practically an obsolete substance commercially and is only so produced where mechanical power is unobtainable.

The "setting" of milk in pans, either shallow or deep, never results in any complete separation of the butter fat. When milk is allowed to stand in cylindrical vessels, it is found that after a definite cream layer is formed, the milk below has at all points a practically constant composition at any moment. To illustrate this the following experiment was carried out by the writers in such a manner that samples of milk could be drawn off at various levels in a cylindrical vessel after various periods.

The sampling tubes were placed so that samples from immediately below the cream layer and at three equal distances between it and the bottom, could be obtained. The following table gives the results:

Fat in original sample	Time	Three lower sampling tubes		
		A	B	C
3.70	2½ hours.	2.70	2.65	2.70
3.70	19 hours.	2.15	2.15	2.20

Another experiment gave similar results:

Fat in original milk	Time	Sampling tubes			
		A	B	C	D
3.40	4 hours.	2.70	2.70	2.75	2.75
3.40	6 hours.	2.70	2.45	2.45	2.55
3.40	24 hours.		2.20	2.20	1.95

The similarity of the fat percentage at the different levels suggested that milks containing an augmented fat content could be easily obtained by gravitation, the process involving only two rough fat estimations. The figures on p. 99 show this to be the case.

Advantage of this has been taken by the writers in the preparation of modified milk for infant feeding.

It is an interesting fact that the first portion of any milking, usually termed "fore milk" is less rich in fat than the last portions called the

Fat in original sample (a)	Fat in lower layers (b)	Fat in enriched milk		Ratio $\frac{a}{b}$
		Found	Calculated	
3.67	2.45	7.1	7.0	1.50
3.62	2.37	6.25	6.0	1.53
3.55	2.20	8.15	8.0	1.61
3.80	2.57	6.65	6.5	1.47
3.60	2.50	7.40	7.0	1.44
3.55	2.47	7.05	7.0	1.44

"strippings." This is well seen in the following table of results obtained by the writers.

	Quantity of milk in cubic centimeters	Fat, %	Total solids, %
Experiment I.—Fore milk	170	3.95	13.64
	340	4.60	14.50
	284	5.60	15.20
Strippings	284	6.30	16.01
Experiment II.—Fore milk	260	1.80	11.12
	300	2.52	11.80
	300	3.20	12.32
Strippings	170	4.00	13.02
Experiment III.—Fore milk	150	0.80	10.24
	150	0.70	10.18
	170	0.70	10.16
Strippings	240	1.42	10.72
Experiment IV.—Fore milk	200	2.22	11.86
	400	2.60	12.24
	450	2.40	12.03
Strippings	460	4.37	13.76
Experiment V.—Fore milk	160	1.90	10.48
	430	3.00	12.12
	240	3.05	12.00
Strippings	130	4.45	13.16
Experiment VI.—Fore milk	330	0.68	10.44
	990	0.55	10.31
	415	0.60	10.33
Strippings	460	0.62	10.32

The rate at which cream rises in milk is dependent on several different factors. This point has been carefully investigated by one of us (*The Dairy*, 1904, 16, 296) in connection with the use of the cream-

ometer as a test for the proportion of fat in milk. The factors are principally (a) the temperature of setting, (b) the effect of mechanical manipulation; both of which tend to decrease the apparent volume of cream thrown up. The addition of water has no effect on the volume of cream, no greater proportion being obtained.

The following table exemplifies the effect of temperature.

Volume of cream at 13-18°	Volume of cream at 36°	Percentage reduction
9.3	4.9	47.3
17.3	11.6	32.9
10.9	5.7	47.7
6.2	4.0	35.5
5.9	3.6	39.0
9.0	6.2	31.1
8.9	5.6	37.1
6.0	5.0	16.6

The want of uniformity between the ratio (R) of percentage volume of cream to actual percentage of fat is very marked. The following figures were obtained for milk set for 4 hours at 25°:

Values of R	Mean	
1.78	1.92	{ Farmers' milk on arrival at depôt after train journey.
2.73		
2.22		
1.46		
1.42		
2.24	2.23	{ Mixed milks before delivery to customers.
2.17		
2.28		
1.67	1.86	{ Mixed milks on return from rounds.
1.92		
2.00		

It will be noted that R becomes much more uniform after mechanical treatment (cooling, cleaning, etc.).

Hunziker has compared the following methods of obtaining cream:

- (1) By the hand separator.
- (2) By deep setting.
- (3) By shallow setting.
- (4) By dilution with water.

He concludes that the loss in butter fat by the last 3 methods, as compared with the first, is respectively 6, 9 and 10 times.

The plea of this natural rising of cream is often submitted as a defence of the deficiency of fat in samples of milk taken by inspectors for Public Health Control. Undoubtedly such deficiency is often so caused in milk allowed to stand in tall vessels on the counters of shops, where the milk is drawn off from time to time by means of a tap at the bottom, and for this reason such vessels should be fitted with a suitable stirrer, so that proper admixture may be made at intervals. In the case of milk carried in tall "churns" on ordinary delivery rounds, there is much less tendency, on account of their shape and movement, to the separation of cream, and there is little difficulty in delivering a uniform milk, even on rounds of 3 to 4 hours.

The ascent of the fat globules when milk is allowed to stand causes no alteration in the composition of the milk, other than the separation of the fat. It has often been erroneously stated that the fat globules carry up with them certain protein constituents, an opinion which has been held to support the view that these globules are surrounded by a membrane of protein. Careful experiments on the composition of milk and of the cream and skim milk after separation, show that the ratio of solids-not-fat to water is the same in each case.

The following exact experiments carried out by H. D. Richmond fully confirm this view:

	Milk	Skim milk	Cream
Sp. gr. ....	1.0308	1.0349	.....
Total solids, % .....	12.75	9.27	34.61
Fat, % .....	4.10	0.28	28.09
Ash, % .....	0.71	0.78	0.58
Non-fatty solids, % .....	8.65	8.99	6.52
Ratio of non-fatty solids to 100 of water .....	9.91	9.91	9.97
Ratio of ash to 100 of water .....	0.81	0.86	0.89

Tatlock and Thomson (*Analyst*, 1910, 35, 5) have published figures showing that the proportion of solids in the non-fatty portion of cream is slightly higher than in the milk from which it was made, but that the difference has no practical significance and may be disregarded. These authors refer to the fact that many years before



the publication of their paper in 1910, deficiency of the non-fatty-solids had been held to indicate the presence of added water. Lerrigo (*Analyst*, 1928, 53, 488) returns to this suggestion, putting forward a figure of 8.7% as the lower limit for non-fatty-solids in the serum of the cream and a figure of 9.2% for cream from average milk. These figures are calculated from the proportions of total-solids and fat in the cream and may be compared with Tatlock and Thomson's figures which range from 8.7% to 10.07% with an average of 9.4%, and the corresponding figure calculated from the above table of H. D. Richmond, viz: 9.97%. Tatlock and Thomson describe a case in which a cream having 7.91% of solids in the non-fatty portion was shown to be watered.

It is necessary to point out that there are several sources of error in connection with such experiments, which seem to have been overlooked by some investigators. These are: (a) the use of too small quantities of milk, (b) loss of water by evaporation during the passage through the separator, (c) "caking" in the pasteurisers, etc.

All cream offered for sale to-day is the product of mechanical separators, and of such machines there is a very great variety on the market, and the adoption of any type of machine is largely a question of the taste and convenience of the purchaser. The earlier "open bowl" type has now been almost entirely displaced by "disc" machines, as this latter type admits of a much more rapid and perfect separation than was possible with the former, though in the opinion of the writers the "open bowl" type threw a cream of smoother consistency and greater freedom from lumpiness. The greater capacity of the disc machine is easily understood from the following illustrations:

In the "open bowl" type, particles of cream or skim milk at the point A would have to travel half the radius of the bowl (in their respective directions) under the effect of the rotation; but in the "disc" type the effective diameter is reduced to the distance between two discs. The fat globule on reaching the upper surface of the disc below then slides to the centre without encountering the resistance of skim milk passing in the other direction, while the corresponding particle of skim milk on reaching the under surface of the disc above, similarly slides down until it reaches the periphery of the separator.

All separators at present on the market are of the central-feed type, but the ideal form would be a separator which fed at the periphery.

The writers have carried out experiments with such a separator and have ascertained that the capacity is enormously increased thereby.

A by-product always obtained during the use of mechanical separators is a substance known as "separator slime." This substance has

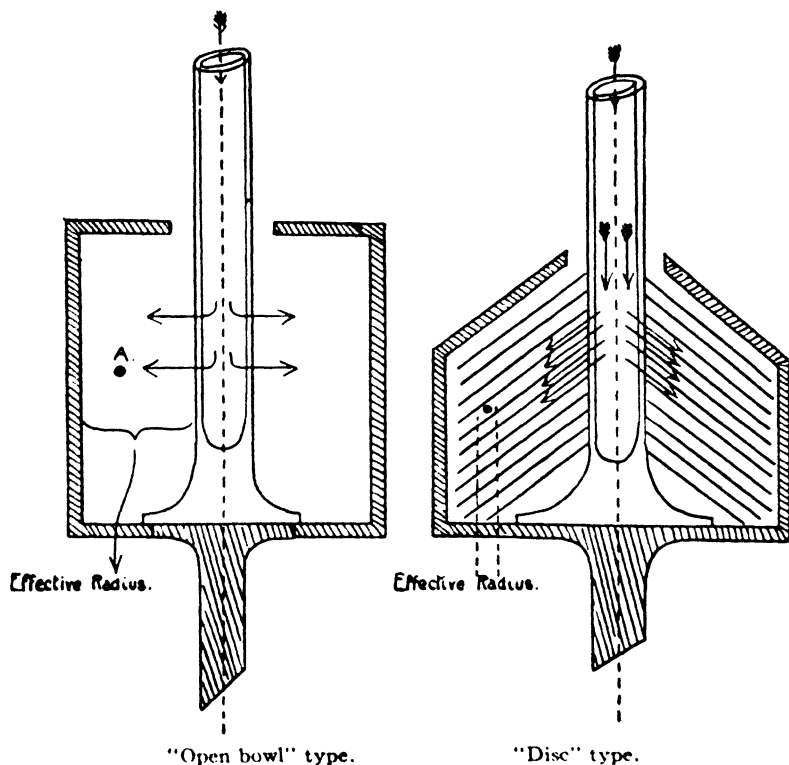


FIG. 1.—Cream separators.

been credited with the most extraordinary compositions, but consists of quite harmless and natural ingredients. It contains any dirt and cellular elements present in the original milk, but the chief component is casein, which, being present in a colloidal state, suffers a slight precipitation under high centrifugal speed. It has been shown by Friedenthal (*Ber.*, 1911, 44, 904) that casein can be separated quantitatively from milk by a speed of 10,000 revolutions per minute.

The analysis shown on p. 104 has been published by H. D. Richmond (*Analyst*, 1894, 19, 86).

Total solids.....	33.76%
Fat.....	0.50%
Lactose.....	0.50%
Casein.....	22.00%
Ash.....	3.01%

Olson (*J. Biol. Chem.*, 1908-09, 5, 261) finds a new protein in the slime after removal of casein and albumin with dilute acids and alkalis. It is very rich in nitrogen (18.93%) and gives the biuret reaction and apparently has the properties of an enzyme.

The quantity of fat lost in the separator slime has been investigated by Gordan (*Milch Zentralblatt*, 1905, 1, 599) with the following results:

Milk in litres	Weight of slime in grm.	Grm. of fat per 1,000 grm. of slime
2,823 unstrained.....	1,200	15.0
2,795 unstrained.....	1,185	15.0
2,794 unstrained.....	980	10.0
3,900 strained.....	715	10.0
3,627 strained.....	720	10.0
3,710 strained.....	740	12.0

Modern mechanical separators are so arranged that a cream of any desired fat percentage from 18 to 60% can be obtained. There is no legal standard in England for the percentage of fat present in marketable cream but trade custom requires a thick cream to contain not less than 50% of fat, while cream for use in the bakery trade usually contains 42 to 43%. On the Continent of Europe, cream with low percentage of fat (20%) is usual; while in America standard cream must not contain less than 18%<sup>1</sup> though an artificial distinction is made between heavy and light creams, heavy creams containing, on an average, about 40% of fat.

The public judges the quality of cream largely by its thickness. This, however, is no true guide to the actual fat percentage. The consistency of cream is dependent on many factors, one of the most important of these being the temperature to which the milk has been exposed before and during separation, and as a preliminary pasteurisation is almost always resorted to in modern separating, it is by no means uncommon to obtain cream, made by an unskilled or negligent

<sup>1</sup> The definitions of the Department of Agriculture of the U. S. A. (15, 11, 1928) require cream to contain not less than 18% of milk fat and not more than 0.2% free acid, calculated as lactic.

operator, containing a large percentage of fat with an appearance sufficiently thin to suggest a much lower percentage, and for this there is little legitimate remedy beyond an excessive and immediate cooling of the cream as it leaves the separator.

Various practices have been in vogue from time to time for the artificial thickening of cream, and where these involve the addition of foreign material they must be regarded as adulteration if their effect is to produce the appearance of a larger proportion of fat than that actually present. The various methods are dealt with under "*Methods of Analysis.*" (Thickening agents.)

Such practices were rendered illegal in England by the Public Health (Preservatives etc. in Food) Regulations of August 4th 1925, and as amended in 1926/27, in which the sale of cream containing any thickening substance is specifically prohibited; in these regulations the sale of any article recommended for use as a thickening substance for cream and the importing of cream containing thickening substances into England or Wales are also prohibited.

### A. (b) CREAM SUBSTITUTES

Substitutes for cream have been manufactured for many years, chiefly for use in the filling of cream buns and cream cakes, etc. a sample of so-called "Jernut cream" examined by the authors gave the following figures:—Total solids 38.01, fat 32.85, protein 2.57, lactose 2.04 and ash 0.55. The fat on examination proved to be a mixture of 85% coconut oil and 15% butter fat. More modern cream substitutes are made by emulsifying various proportions of refined vegetable oils and hydrogenated oils with dried milk and water, or with skimmed milk. In such substitutes while the proportion of fatty matter may be found to be anywhere between 30 and 80%, it is generally in the neighbourhood of 40%.

Such cream substitutes keep fresh for a considerably longer time than cream and, in consequence, their use was largely extended after the introduction in England of the Preservative Regulations of 1925. These regulations also led to the production to a much larger extent than before of "*artificial cream*"—a product which is legally defined (*vide infra*) and must not be confused with cream substitutes containing ingredients not derived from milk, which must not be described as "*artificial cream.*"

**A. (c) ARTIFICIAL CREAM (OR RECONSTITUTED CREAM)**

In 1929 an Act was passed to regulate the sale and manufacture in Great Britain of Artificial Cream, which is defined therein as "*an article of food resembling cream and containing no ingredient which is not derived from milk except water or any ingredient or material which by virtue of the proviso to subsection (2) of section 2 of the Foods and Drugs (Adulteration) Act 1928, may lawfully be contained in an article sold as cream.*" The Act<sup>1</sup> provides that where any substance purporting to be cream or artificial cream is artificial cream, it shall not be sold under a description including the word "cream" unless that word is immediately preceded by the word "artificial." Further, receptacles used for conveying artificial cream or for containing it when exposed for sale must be labelled with the words "*artificial cream*" in large and legible type. Premises where artificial cream is manufactured or sold, with certain specified exceptions, must be registered.

It is considered that this Act, and the Foods and Drugs (Adulteration) Act of 1928, are sufficient to prevent the sale of cream substitutes made from ingredients foreign to natural cream, such as are described on p. 105 as cream or artificial cream. In accordance with this legal definition "*artificial cream*" may be, and is usually, prepared by emulsifying together butter, dried milk and water, or by emulsifying other substances manufactured from milk in such a way as to produce a material resembling cream in appearance, flavour and composition.

In the Ministry of Health Circular No. 989, it is recognised that, if due precautions be taken in blending the ingredients of artificial cream, it cannot be distinguished analytically with any certainty from cream. Tests which have been proposed for so distinguishing between the two products will be dealt with in the section on analysis.

**A. (d) TINNED CREAM**

A considerable quantity of cream is now sterilized and tinned. Such cream usually contains about 25 to 26% of milk fat, and it appears that by the methods of manufacture at present employed this proportion cannot be exceeded without risking the production of a lumpy or unattractive product.

<sup>1</sup> Ministry of Health Circular, No. 989.

**A. (e) CLOTTED CREAM**

This product is largely associated in England with Devonshire and Cornwall, though there is no difficulty in preparing the substance anywhere, provided milk fresh from the cow can be obtained. The mode of preparation is usually as follows: Whole milk, warm from the cow, is placed in pans about 15 in. in diameter and 6-7 in. in depth, with sloping sides; the milk is allowed to stand in these for 12-24 hours, and they are then placed in the scalding stove and brought to a temperature of 175-180° F. in not less than half an hour. They are then removed and allowed to cool naturally. The pans are then placed on a revolving table, and the cream removed with a "slice."

From the method of preparation it follows that the ratio of solids-not-fat to water is altered by the evaporation that takes place, the solids-not-fat being higher than in separated cream.

The results of Vieth and Richmond, for an aggregate number of 463 samples of clotted cream analysed during the series of years 1886 to 1893 inclusive, show the following range of composition:

	Water, %	Total solids, %	Fat, %	Solids not fat, %	Ash, %
Maximum.....	45.57	74.84	68.59	11.70	1.17
Minimum.....	25.16	54.43	45.78	5.26	0.42
Average.....	34.56	65.44	58.05	7.39	0.58

The average figure found by Richmond for clotted cream produced in 1896 were: total solids, 67.64; fat, 59.16; solids not fat, 8.48; and ash, 0.68%.

The analytical methods for clotted cream are exactly similar to those employed for the analysis of cream (page 108). Search should be made for preservatives and thickening agents as the former are illegal and the latter are quite unnecessary in properly prepared clotted cream.

**A. (f) SKIM MILK**

*Skim milk* is the lower layer, comparatively poor in fat, which remains when the cream is removed by skimming or similar means. It may be regarded as essentially new milk deprived of the greater part of its fat.

Some analysts have attempted to draw a sharp distinction between skimmed milk and separated milk, on the ground that the latter product commonly contains a smaller proportion of residual fat than hand-skimmed milk. The distinction should be borne in mind, but the two products are merely varieties of the same article produced by different processes.

The composition of skim milk, as affected by the various processes for its production, is shown by the following results of Vieth (*Analyst*, 1884, 9, 63).

No.	Sp. gr.	Total solids, %	Fat, %	Solids not fat, %	Remarks on system
1	1.0350	9.75	0.55	9.20	Shallow pans.
2	1.0355	9.90	0.54	9.36	
3	1.0340	10.10	1.00	9.10	
4	1.0355	10.43	0.98	9.45	
5	1.0335	9.68	1.05	8.63	Deep pans.
6	1.0345	9.70	0.60	9.10	
7	1.0355	9.81	0.43	9.38	
8	1.0350	10.26	0.88	9.38	
9	1.0365	9.96	0.46	9.50	Centrifugal system.
10	1.0350	9.28	0.34	8.94	
11	1.0370	9.94	0.34	9.60	
12	1.0370	9.80	0.35	9.45	

A much greater skimming effect is brought about with modern separators, and in good working not more than 0.05–0.10% of fat should be found.

### Analytical Examination

**Analysis of Cream.**—The analysis of cream is undertaken (1) for the detection of added substances, in which case exact methods are required, or (2) for control of separators for which simpler methods are employed. Cream is also examined for the presence of preservatives and thickening agents.

**Fat.**—*Rapid estimation for control.*

(1) *The Babcock Method.*—Approximately 10 grm. of well-mixed cream are placed in a special "bottle," a convenient balance being made for the purpose and usually employed. 5 to 6 c.c. of water are added, and then 17.5 c.c. of the regular sulphuric acid (sp. gr. 1.82–

1.84) measured in and the test continued as under milk. The percentage of fat is calculated by the following formula:

$$\% \text{ Fat} = \frac{\text{Reading} \times 18}{\text{weight of cream taken}}$$

(2) *The Gerber Process.*—This is similar to the above in certain ways, a different bottle being employed. The writers recommend the use of the ordinary Gerber-test bottle and not the special form made for the purpose. About 1 grm. of cream is weighed out in a small funnel fitted with a ground glass rod and suspended from the balance hook (see Fig. 2). The cream is washed into the bottle with 2 quantities of 5 c.c. of warm water, and the bottle being held in a *slanting* direction, the liquid is caused to fill the bulb by tapping, if necessary. The contents are cooled and 10 c.c. of sulphuric acid (sp. gr. 1.820–1.825) run in and 1 c.c. of amyl alcohol. The bottle is shaken as usual and allowed to stand in water at 65–70° for 5 minutes. After rotation the volume of fat is read *without* warming the tube. The percentage of fat is calculated by the following formula:

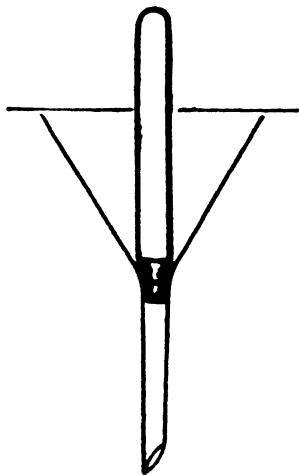


FIG. 2.—Cream funnel.

$$\% \text{ Fat} = \frac{\text{Reading (cold)} \times \text{factor}}{\text{weight of cream used}}$$

The usual factor given = 11.22, but the writers find that the factor is variable and dependent on the fat percentage. They have estimated the following factors:

55% fat.....	10.81
45% fat.....	11.23
20% fat.....	11.41

It is, however, advisable to determine a personal factor if many estimations are being made.

Gerber tubes of special construction are now made which obviate the necessity of using this factor, and offer certain advantages in manipulation, especially in routine working. A certificate of standardisation by the National Physical Laboratory may be obtained, if desired.



(3) *The Mats-Weibull Method.*—For the rapid control of cream the method of Mats-Weibull as modified by L. Fr. Rosengrew is both useful and satisfactory. The total solids are estimated and the fat is calculated from the formula,

$$f = 1.1t - 9.5$$

where  $f$  is the required percentage of fat and  $t$  is the total solids as estimated. The total solids may be estimated sufficiently exactly for this purpose by carefully boiling off the water in a porcelain dish over a free flame exactly as in the rapid estimation of water in butter by Patrick's method (Vol. II, p. 393). When carefully carried out, the results are quite concordant with those obtained by drying in a water-oven. This of course assumes that no water has been added to the cream. If water has been added, the amount can be calculated fairly exactly from the formula,

$$\% \text{ added water} = \frac{100f + 950 - 110t}{0.95t - f}$$

where  $f$  = the estimated percentage of fat

and  $t$  = the estimated total solids.

**Fat.**—*Exact estimation by the Gottlieb process.*

From 0.5 to 1.0 grm. of the cream is weighed out direct into the apparatus shown in the sketch.

This weight is made up to 5.2 grm. with water. To the mixture is added 0.5 c.c. of ammonia (sp. gr. 0.925), and the contents mixed by rotation, 5 c.c. strong alcohol (95% by volume) are added and the contents again mixed by rotation after which about 12.5 c.c. of methylated ether are added and the contents of the flask mixed by inversion 3 times after closing with a tight fitting india-rubber stopper; 12.5 c.c. of petroleum spirit (redistilled and boiling between 40° and 60°) are then added and the contents mixed by inversion. The flask is allowed to stand for 15 minutes and the mixed ethers, down to within 0.5 cm. of the lower layer, blown off into a tared flask with the wash bottle arrangement shown. This is then carefully removed and the under surface of the india-rubber stopper and the sides of the flask washed

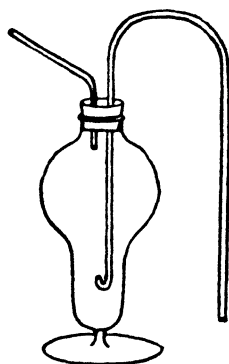


FIG. 3.—Rose-Gottlieb apparatus.

down with about 20 c.c. of mixed ethers (recovered from previous tests), the apparatus being rotated to mix up the ether. After a few minutes settling the ether is blown off as before into the flask and the washing repeated once more with 20 c.c. of mixed ethers. (It is unnecessary and even inadvisable to mix the contents of the apparatus with the washings *by inversion*.) The ether in the tared flask is distilled off, and the fat dried at a temperature not exceeding  $100^{\circ}$  to constant weight, a state usually attained in half an hour. The method on p. 138 may also be employed.

The following is the form in which this method is recommended by the A. O. A. C. (Bulletin 107):

One grm. of cream is placed in a glass cylinder about 2 cm. diameter and 36 cm. long (a 100 c.c. burette or eudiometer will do), 1 c.c. of concentrated ammonium hydroxide added and mixed. Then in succession are added 10 c.c. alcohol (92%), 25 c.c. washed ether, and 25 c.c. petroleum spirit (b. p. below  $80^{\circ}$ ). The cylinder is closed with a moistened cork and shaken after each addition of the above liquids. It is allowed to stand 6 hours. The clear liquid is pipetted off into a tared flask by means of a siphon, with the end drawn to a fine point and lowered into the solution within 0.5 cm. of the bottom layer. After evaporating the solution in a hood the flask is dried in a steam oven for 2 or 3 hours and weighed. When the sample is high in fat a second treatment with 10 c.c. of ether and petroleum spirit is advisable.

**Total Solids.**—These are determined by the method on page 137 using 1.5 grm. of cream and 5 c.c. of water.

**Aldehyde Figure for Cream.**—Richmond<sup>1</sup> has pointed out that a determination of the aldehyde figure for cream will at once show whether a low fat percentage is due to the addition of milk or water.

The aldehyde figure for cream devoid of fat is practically identical with that of skim milk, so that the addition of milk to cream has no effect on the aldehyde figure calculated on the cream devoid of fat, whilst the addition of water naturally at once lowers it.

The aldehyde figure is determined in the usual way and calculated to c.c. of  $N/1$  strontium or sodium hydroxide per 1,000 c.c. of cream. This figure is then calculated to cream devoid of fat thus:

$$\text{Aldehyde figure} \times \frac{100}{100 - \text{fat}}$$

<sup>1</sup> *Analyst*, 1914, 39, 243.

The average figure for cream is 20.8 c.c., using  $N/10$  strontium hydroxide for the titration. If  $N/10$  sodium hydroxide be used the figure becomes 15.8° c.c. If the figure obtained is distinctly below 20.8 c.c. (or 15.8 c.c. for sodium hydroxide) the addition of water is indicated.

The above considerations also bear out the contention of Richmond that the solids not fat of cream are in the same ratio to the water present as they are in milk.

In determining the aldehyde figure, Richmond has suggested the use of a standard pink colour for the detection of end points. The standard tint is made by adding to 10 c.c. of milk 1 drop of 0.01% solution of rosaniline acetate in 96% alcohol.

It follows that the acidities determined in this manner will be slightly higher (about 1.5°) than those obtained in the usual manner by titrating till the first pink tinge is observed to be permanent, but the titration for the aldehyde figure, which is a difference only, is not affected and is probably rendered more accurate as the sensitiveness of the observer's eye to a pink colour varies in different individuals.

**Nitrogen.**—This is best estimated by Kjeldahl's method in the usual way; but a preliminary extraction of the fat is necessary or severe charring results. To effect this, the water in the cream is rapidly removed, by placing the digestion flask in warm water and connecting to a vacuum pump till the water is removed. Ether is then added and poured off through a small filter paper, the treatment being repeated and the filter paper finally dropped back into the flask.

**Lactose and ash** are estimated as in MILK.

### Preservatives

In Great Britain preservatives are not allowed in cream; the following methods are suitable for their detection. (See also under MILK.

**Boron Compounds.**—About 20 c.c. or less of the cream are made slightly alkaline with sodium carbonate and boiled down rapidly in a platinum dish till deep brown, and the fat poured off. The residue is burnt till only carbon remains, acidified with hydrochloric acid and after insertion of turmeric paper, carried to dryness. In the presence of boron compounds the paper will take on shades of pink to rose-red,

varying with the quantity present, the colour being changed to indigo blue by a drop of strong ammonia.

**Fluorides.** (a) *In the Presence of Boron Compounds (Hehner's Method).*—50 grm. of cream are made just alkaline with sodium carbonate and rapidly boiled down in a platinum dish, the fat poured off, and the residue charred well. The contents of the dish are taken up with hot water and 1 grm. of calcium chloride dissolved in a little water added, and a moderate excess of sodium carbonate then run into the hot solution. The contents of the dish are filtered and the filter dried and burned completely in a platinum dish. The residue is taken up with acetic acid (1 in 3) and well boiled for 5 minutes, the dish being covered. The residue is filtered off and ignited, moistened with a few drops of conc. sulphuric acid, and the dish, covered with a waxed watch-glass having some marks scratched through the wax, is heated on a hot plate (80°) for half an hour, the watch-glass being filled with water or ice as necessary. After removal of the wax, the glass is examined for etching.

(b) *In Absence of Boron Compounds.*—The residue after burning off the fat, is burned nearly white and treated direct with the sulphuric acid as under (a).

**Benzoic and Salicylic Acids.**—50 grm. of the cream are diluted in a flask with 100 c.c. of water and made alkaline with sodium carbonate. The flask is heated in boiling water for 5 minutes and 5 c.c. of 10% calcium chloride added to the hot liquid (the heating being continued till the protein and fat separate). The flask is cooled and the contents without mixing filtered into another flask. The filtrate is neutralised to litmus paper and treated with 10 c.c. of Fehling's copper sulphate solution and then with 10 c.c. of potassium hydroxide solution (31.15 grm. per 1,000 c.c.), mixed and filtered into a separating funnel. The clear solution in the funnel is acidified with dilute sulphuric acid and extracted with 20 c.c. of ether, adding a few drops of alcohol if necessary. The ether is separated and washed twice with a little water and then, after the addition of 5 c.c. of water and a drop of phenolphthaleïn, saturated solution of barium hydroxide is run in till, on shaking well, the pink colour is permanent. The aqueous layer is filtered off and concentrated to 2–3 c.c., filtered again if necessary into a small test-tube, sufficient very dilute acetic acid added to discharge the pink colour and then 1 drop of neutral ferric chloride solution. A violet colour indicates salicylic

acid, while in the presence of benzoic acid, a flesh-coloured turbidity or precipitate forms, which does not dissolve on the addition of a further small quantity of ferric chloride solution.

**Peroxides.**—Solutions of hydrogen peroxide are used for preserving cream, but usually the amount added will have completely decomposed before the cream is examined. If present it may be detected by the addition of a 1% solution of titanous acid in 1:3 sulphuric acid. In the presence of peroxides a yellow colour is developed, which is best seen if the reagent be allowed to flow down the side of the test-tube containing the cream.

**Formic Acid.**—As preservatives containing formic acid mixed usually with glucose syrup have been sold, the examination for this substance is important. 100 grm. of cream are mixed with 100 c.c. of water and 20 c.c. of 20% phosphoric acid and steam distilled till 200 c.c. approximately have passed over, the end of the condenser being allowed to dip under the surface of a mixture of 20 c.c. of lime water (containing at least 1 gm. of lime in suspension) to which has been added 2 c.c. of 3% acetic acid free from formic acid. The distillate is evaporated to dryness and scraped into a small hard glass tube about  $\frac{1}{2}$  in. in diameter, closed at one end, the other being drawn out and passed into one limb of a miniature U-tube containing 1 or 2 c.c. of water, care being taken that none of the water can be sucked up into the hard glass tube; the contents of the tube are then heated strongly till nothing more distils. The water in the U-tube is then mixed with 2–3 c.c. of Schiff's reagent, when a violet colour will appear either at once or within 30 minutes if formates were present in the cream. A very slight colour is often given by pure cream but could not be mistaken for the colour produced even by 1 part in 1,000 of formic acid. Schiff's reagent is prepared by dissolving 0.5 grm. of rosaniline hydrochloride in about 50 c.c. of water. A mixture of 10 c.c. of saturated sodium hydrogen sulphite solution, and 5 c.c. hydrochloric acid (conc.) are then added and the whole made up to 500 c.c. This solution keeps well in the dark.

**$\alpha$ - and  $\beta$ -Naphthol.**—A few cubic centimetres of cream are diluted somewhat and cleared with calcium chloride as in the case of benzoic acid and the filtrate, after being made slightly alkaline with sodium hydrogen carbonate, mixed with a few drops of an emulsion of  $\alpha$ -naphthionic acid, when in the case of  $\alpha$ - or  $\beta$ -naphthol or Abrastol a fine crimson colour *immediately* develops (only an immediate colour is of any importance).

The  $\alpha$ -naphthionic acid is prepared as follows: About 0.2 grm. of 1-4 naphthylamine sulphonic acid are boiled with 10 c.c. of 50% alcohol, cooled in ice, 1 c.c. of 1 in 3 sulphuric acid added *gradually*, and then slowly 1 grm. of potassium nitrite dissolved in 10 c.c. of water (the suspension turns a yellowish colour—if pink, the nitrite is being added too fast). The mixture is allowed to stand for 2 or 3 minutes in the ice water, the precipitate filtered off, washed with 2 or 3 c.c. of water and then emulsified with about 5 c.c. of water, and used as above directed.

**Formaldehyde and Hexamethylene Tetramine.**—These are tested for by Hehner's method. In the presence of small quantities of formalin a violet ring develops at the junction of the acid and the cream at once, and after a short interval in the case of hexamethylene tetramine.

**Mystin.**—A preservative, under the name of "Mystin," which seems to be a mixture or compound of formalin and a nitrite, has been offered to the Dairy Trade.

It does not give the ordinary reactions for formalin. The method proposed by Monier-Williams in a report on this preservative to the Local Govt. Board (London) is perhaps most reliable and simple. 5 c.c. of the cream (or milk) are mixed with 5 c.c. of water and to it are added 0.5 c.c. of a 10% solution of urea, then 1 c.c. of  $N/H_2SO_4$  and the mixture heated in boiling water for 2 minutes. The mixture is then cooled and the ordinary test for formaldehyde made with sulphuric acid. If the preservative has been recently added—whey obtained from the cream on acidification with sulphuric acid will give a blue colour with diphenylamine.<sup>1</sup>

**Quantitative Estimation of Preservatives.**—The only preservatives that need be estimated with accuracy are boron compounds and salicylic acid.

**Boron Compounds.**—20 grm. of the *well mixed* cream are placed in a 100 c.c. flask, mixed with 2 c.c. of 10% sodium carbonate and diluted to the mark with water and 10% calcium chloride solution added in the following proportions,

For 55% fat.....	12.5 c.c.
For 50% fat.....	11.4 c.c.
For 40% fat.....	9.3 c.c.
For 30% fat.....	7.2 c.c.

<sup>1</sup> A solution of formaldehyde containing potassium chlorate is also sold.

these volumes compensating for the precipitated fat and protein. The mixture is warmed till precipitation has taken place, cooled and filtered. 20 c.c. of the filtrate are mixed with 10 c.c. of a 0.5% solution of phenolphthalein in 50% alcohol. The mixture is boiled and titrated while boiling with  $N/10$  sulphuric acid till distinctly acid, boiling for 15 minutes and then titrating with  $N/10$  sodium hydroxide till pink and the end point finally adjusted, while boiling, by the alternate use of the acid and alkali. To the solution are now added 2 grm. of mannitol (or glycerol to make one-third of the final volume) and the solution titrated with  $N/10$  sodium hydroxide till pink.

If  $x$  = c.c. of alkali used for the final titration and  $y$  = c.c. required by the mannitol or glycerol then

$$(x - y) \times 0.0062 \times 25 = \text{boric acid in \%}.$$

(The above is a modification of the method of Richmond and Miller, *Analyst*, 1907, 32, 144.)

**Salicylic Acid.**—The estimation of this preservative is difficult on account of the solubility of the acid in fat, and any method of clearing the cream in acid solution gives erroneous results. It may be estimated with accuracy by the method of Revis and Payne (*Analyst*, 1907, 32, 286).

### Thickening Agents

(1) **Saccharate of Lime.**—To 10 c.c. of the cream in a test-tube add 0.5 c.c. of strong hydrochloric acid and 0.1 grm. of resorcinol dissolved in 5 c.c. of water. The mixture after shaking is placed in boiling water for 5 minutes, when in the presence of saccharate of lime a pink to deep red colour gradually develops. Pure cream turns yellowish or brown. This test is strictly one for the presence of cane sugar, but any attempt to determine the presence of lime saccharate by an increase in the calcium content of the ash is likely to be erroneous.

(2) **Gelatin (Stokes' Method).**—10 c.c. of cream are mixed with an equal quantity of Wiley's dilute acid mercuric nitrate solution which has been diluted with twice its volume of water, allowed to stand for 5 minutes and filtered. The filtrate is mixed with an equal volume of saturated aqueous picric acid. Gelatin produces a yellow precipitate.

(3) **Gums (Including Agar-agar).**—About 50 grm. of cream are diluted with 100 c.c. of water, heated in boiling water, and cleared by the addition of 5 c.c. of 10% calcium chloride solution.

The mixture is filtered clear, for preference in a hot water funnel, cooled, and from one-half to two-thirds of its volume of strong alcohol added. The precipitate is filtered off and boiled with a small quantity of water till no more dissolves, filtered and evaporated down to 5 c.c. when the solution will gelatinise on cooling in the presence of agar-agar. An examination for diatoms is not satisfactory.

If a mixture of gelatin and gum are suspected, they may be separated as follows:

The *boiling* dilution of the cream is cleared as before, and filtered *clear*, the filtrate precipitated with alcohol and a solution of the precipitate obtained as above. To a few cubic centimetres of the filtrate picric acid solution is added, when a turbidity or precipitate is produced if gelatin is present. In this case the remainder of the filtrate from the cream is evaporated to about 25 c.c. and a 10% solution of tannin added, until a precipitate ceases to form. To the mixture, which must be under 60° are added 5 to 10 c.c. of white of egg and the whole heated in boiling water for 30 minutes. The solution is filtered hot and concentrated on the water bath to a small bulk, when the presence of gum is indicated by gelatinisation. Jacobs and Jaffe (*Ind. Eng. Chem. [Anal. Ed.]*, 1931, 3, 210) point out that this method is a general method for the detection of gums and is not specific for agar-agar. They describe in full a method for the identification of gums by group reagents which is readily applicable to cream and other milk products.

(4) **Casein, Dried and Condensed Milks.**—The addition of these substances can usually be detected by an unusually high solids-not-fat. Casein, however, may generally be found by diluting the cream with an equal volume of boiling water and strongly centrifuging the mixture, when a gummy mass of undissolved casein is usually obtained. Cream thus treated is in most cases frothy, on account of the agitation employed in incorporating the thickening material.

(5) **Pectoses.**—This class of substances may be detected in the following manner:—10 to 20 grm. of the sample are treated exactly as in the Gottlieb process for fat estimation, increasing the reagents in proper proportion. The process is best carried out in a graduated cylinder. The aqueous layer, which, if pectoses are present is



usually characterised by containing flocculent particles, is filtered, or better centrifuged, and the gummy deposit washed well with water, either on the filter or if a rotator be used, by shaking with water and repeated rotation. The deposit is then mixed with water to a volume of 5 c.c., one c.c. of conc. hydrochloric acid added and the mixture heated in boiling water for 45 minutes. The mixture is then cooled and neutralised with solid carbonate, heated to boiling and poured into 5 c.c. of boiling Fehling's solution. If any appreciable amount of reduction takes place the presence of pectoses in the original sample may be taken as certain. It is essential that all lactose shall have been washed out of the deposit before the acid conversion.

(6) **Starch.**—This substance is easily detected in the usual manner by iodine solution, care being taken to add sufficient iodine to develop a distinct yellow colour before deciding on the absence of starch.

(7) **Homogenisation.**—This is a form of mechanical thickening sometimes resorted to especially in the case of sterilised creams; it is, however, neither permanent nor satisfactory. It can be detected by microscopical examination after well diluting the cream.

### Detection of Artificial Creams

Tests have been proposed for the recognition of artificial creams, based upon the difference between the ease of solution by organic solvents of the fat from this product and from natural cream. These methods can only be regarded as giving an indication of the nature of the product tested and have not yet been developed to a stage which permits of absolute reliance being placed upon the results. At present more certain information is likely to be obtained from a consideration of the relation between the proportions of water and of non-fatty solids which, as has already been pointed out, is the same in cream as in normal milk, but which is often different in the artificial product (compare pp. 101, 102).

### SECTION B. (a) BUTTER MILK

This term is applied to the liquid which runs away from the churn after butter formation has taken place. Its constituents are naturally those of the original sour milk or cream employed for the churning less the greater percentage of the fat. The amount of

the latter varies somewhat with the skill of the operator and the perfection of the souring process, but 0.5% should not be exceeded in proper working.

Butter milk at the present day is only obtained from the churning of cream, as whole milk is scarcely ever used for the production of butter, and as the cream will in all probability have been "soured" it contains variable quantities of lactic acid and consequently has a distinct acid flavour. Sweet cream is but rarely churned as butter formation is in such cases incomplete and only obtained with difficulty. The composition of butter milk closely follows that of milk, except that a portion of the lactose has been converted into lactic acid and there may be a certain amount of protein degradation. On analysis butter milk often appears to be watered, owing to the fact that the "butter grains" are washed in the churn with cold water which is usually run off into the butter milk. The legality of this operation must be left an open question, as the butter factory looks on butter milk as a waste product. The writers are of the opinion that in cases where butter milk is sold ostensibly for dietetic or toilet purposes, it should be undiluted. The analytical methods are those of peptonised milk (see page 150).

Siegfeld and Keisten (*Molkereizeitung Hildesheim*, 1910, 24, No. 48) draw attention to the fact that the Gottlieb process yields usually 0.15-0.25% more fat than the Gerber process and attribute it to a partial homogenisation of the fat during churning.

The following analyses of butter milk are due to Vieth (*Analyst*, 1884, 9, 63):

No.	Total solids, %	Fat, %	Non-fatty solids, %	Ash, %
1	9.77	1.00	8.68	0.69
2	9.03	0.63	8.40	0.70
3	10.39	0.78	9.61	.....
4	8.02	0.65	7.37	1.29
5	9.64	2.51	7.13	0.64
6	8.13	0.82	7.31	0.64
7	10.14	0.92	9.22	0.73
8	8.91	0.50	8.41	0.71
9	8.98	0.40	8.49	1.32
10	10.70	0.54	10.16	0.82
11	9.80	0.76	9.04	0.73
12	9.72	0.80	8.92	0.73

**B. (b) WHEY**

This term is strictly applied to the liquid obtained by straining off the curd produced in milk by the action of rennet. A similar liquid is also obtained when the curd is precipitated by acids or natural souring but the chemical composition of this latter differs from that of a rennet whey.

Rennet, containing as it does both chymosin and pepsin, not only precipitates the curd as paracasein, but produces a partial though slight digestion and a certain proportion of a soluble caseose appears consequently in the whey. The amount of this latter will depend on the time allowed before removing the curd and also on the temperature at which the curd is kept.

In the case of acid precipitation, the casein alone is removed and no caseoses are found. Further, in rennet coagulation a considerable quantity of the calcium of the original milk is left bound with the

**ACID CASEINS**

No.	Water, %	Total solids, %	Fat, %	Ash, %	N <sub>2</sub> in total solids, %	Calc. factor for nitrogen to casein
1	7.80	92.11	0.075	0.00	14.37	6.40
2	1.65	98.35	0.005	0.00	15.33	6.41
3	6.73	93.27	0.055	0.00	14.50	6.45
4	8.65	91.35	0.005	0.00	14.28	6.40
5	5.55	94.45	0.005	0.00	14.71	6.41
6	9.62	90.38	0.090	0.49	14.03	6.40

All were snow-white powders.

**RENNET CASEINS (Paracaseins)**

No.	Water, %	Total Solids, %	Fat, %	Ash, %	N <sub>2</sub> in total solids, %	Calc. factor for N <sub>2</sub> into casein
1	0.61	99.39	0.13	6.85	14.53	6.35
2	0.60	99.40	0.55	7.35	14.31	6.39
3	10.85	89.15	0.25	7.75	12.77	6.35
4	2.97	97.03	0.08	8.55	13.91	6.35
5	7.30	92.70	0.12	6.90	13.42	6.38
6	0.70	99.30	0.10	5.00	14.83	6.38

All were white powders.

curd, while in acid precipitation, the curd is practically free from mineral constituents

In the tables on page 120 there is a collection of analyses of pure acid and rennet curds given by Burr (*Milch. Zentralbl.*, 1910, 6, 383):

One hundred parts of rennet curd ash gave as a mean value— $P_2O_5$  60.64%, Ca 37.44%, Mg 0.088%. The ash in the paracaseins is naturally higher, as some of the ash constituents of the milk are in chemical combination with it.

It must be pointed out that in whey made from very fresh milk and in which the curd is separated at once after coagulation, such as would be the case in the manufacture of sweet whey for dietetic purposes, the percentage of proteins and fat is generally higher than that of whey obtained in the manufacture of cheese.

The constituents of whey are: lactose, lactalbumin, caseoses, generally a small proportion of lactic acid and the bulk of the salts of the milk, and traces of fat. The analytical methods are those of peptonised milk (page 150). For a dissertation on sp. gr. and refraction of serum of milk obtained by precipitation of the curd with calcium chloride see Weigner (*Milch Zentralbl.*, 1909, 5, 473 *et seq.*).

### B. (c) MILK SUGAR (LACTOSE)

Milk sugar is usually manufactured from the whey obtained in the process of cheese manufacture. The following details give the outline of the method usually employed: The whey is acidified with about 2 or 3% of lactic acid and heated to boiling, when the albumin is precipitated and is filtered off, and a clear filtrate containing the sugar and milk salts is obtained. The clarified whey is concentrated in vacuum pans at about 60°, till the solids are nearly 60%, when the pans are emptied into crystallising vats cooled by water. After several hours' standing the thick mass is stirred and again left when a granular yellow pulp settles out. This is freed from the mother-liquor in a hydro-extractor and the liquors are heated to boiling, strained and again evaporated in the vacuum pan and allowed to crystallise, the first and second crops of crystals being mixed. The crude sugar or "sand" is refined by dissolving the mass in water till a liquor of 13–15° Baumé is obtained, mixed with bone-black and a small quantity (0.2%) of acetic acid and heated to 90°, a little magnesium sulphate added and the whole heated to boiling. The liquid is then passed through a filter-press, concentrated in the

vacuum pan to 35° Baumé and run into crystallising tanks, the mother liquors being worked up again for fresh crops of crystals. The mass of crystals is freed from liquid in the hydro-extractor and dried by warm air. The yield is usually about 2.5% of the whey used. Sometimes the crystallisation is hastened by continuous stirring of the concentrated liquors, the sugar being then obtained as a powder.

Milk sugar is still, however, manufactured in certain places by boiling down the whey in open vessels to the crystallising point, a very impure "sand" being so obtained which has to be refined.

The chemistry of milk sugar is fully dealt with in Vol. 1, p. 456.

**Raw Milk Sugar.**—The composition of raw milk sugar is distinctly variable. The following figures have been published by Burr and Berberich (*Milch Zentralblatt*, 1911, 7, 241).

#### RAW MILK SUGAR FROM ONE FACTORY

	Water, %	Total solids, %	Lactose, %	Total ash, %	Protein, N × 6.3, %	Fat, %	Acidity as lactic acid, %	Other constitu- ents, %
Mean.....	1.07	98.93	92.50	1.66	2.07	0.52	0.40	1.81
Maximum.....	2.33	99.82	95.29	2.21	2.95	0.80	0.59	5.09
Minimum.....	0.18	97.67	89.25	1.19	1.32	0.25	0.09	0.03

#### RAW SUGAR FROM VARIOUS SOURCES

	Water, %	Lactose, %	Protein N × 6.3, %	Fat, %	Total ash, %	Water soluble ash, %	Water insoluble ash, %	Acidity as lactic acid, %
Mean.....	2.34	89.60	1.06	0.19	2.70	0.65	2.06	0.44
Maximum.....	14.07	95.95	3.22	0.42	5.44	1.32	4.02	2.14
Minimum.....	0.25	78.45	0.62	0.08	1.23	0.06	1.08	0.07

The ash of raw milk sugar contains: calcium, magnesium, phosphoric and sulphuric acids, but of course will vary slightly with the method of manufacture. The following percentage composition is given by the above authors (mean of 15 analyses):

CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>4</sub>	Cl
30.5	4.5	0.55	3.72	6.10	0.99

**Refined Milk Sugar.**—For the control of refined milk sugar the following examinations are recommended by them:

*First.*—For the presence of cheaper sugars, such as cane, beet, and invert, and for starch.

*Second.*—For the presence in the ash of weighty inorganic substances, such as chalk and gypsum.

*Third.*—For its solubility in hot water—a 1:1 solution of refined milk sugar should be clear and colourless—if turbid, impurities are present.

*Fourth.*—For its nitrogen content.

*Fifth.*—For free acid or alkali.

*Sixth.*—For the presence of other soluble substances.

Burr and Berberich give the following analyses of refined milk sugar from one manufactory:

REFINED MILK SUGAR

	Water	Total solids, %	Total ash, %	Fat, %	Nitrogenous substances, %	Solubility tests at 22 to 23° according to the German Pharm., IV (see below)	Solubility in hot water 1 in 1
Mean . . . .	0.07	99.93	0.08	Negligible	Negligible	0.0358	Clear and colourless.
Maximum . .	0.11	99.98	0.13	Negligible	Negligible	0.0380	Clear and colourless.
Minimum . . .	0.02	99.89	0.05	Negligible	Negligible	0.0330	Clear and colourless.

The mean of 15 ash-constituent estimations gave

CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>4</sub>	Cl
22.50%	0.08%	0.00%	3.59%	20.57%	0.00%

An examination of a number of samples from other factories gave very similar results, except that the nitrogen varied from traces to 0.06% and with some samples there was a slight opalescence of the 1 in 1 hot water solution and the aqueous solution of the sugar when mixed with ammonium sulphide varied in colour from colourless to dark brown. It must be understood that slight traces of iron and copper derived from the piping and vacuum pans may appear in refined milk sugar.

The presence of more than a trace of nitrogenous substances must be looked upon as deleterious, as milk sugar is used simply in solu-

tion for infants' feeding in cases of acute intestinal toxæmia, and organisms readily develop in solutions containing more than traces of protein, which would vitiate the effect of the treatment.

**Pharmacopœial Tests.**—The following are some of the tests which have been suggested from time to time for examining milk sugar for pharmaceutical purposes.

The edition of the British Pharmacopœia still in force gives the following tests for examining milk sugar:

Five grm. dissolved in water should require for neutralisation not more than 1.5 c.c. of  $N/10$  solution of sodium hydroxide (limit of acidity). When 5 grm. are well shaken with 20 c.c. of alcohol (90%) and filtered, the filtrate should leave no appreciable residue on evaporation (absence of sucrose).

The following details are from the French Codex 1927:

*Sp. gr.* 1.534. The commercial product does not lose water of crystallisation at  $150^{\circ}$ , but does at  $170^{\circ}$ , becoming coloured. Melts at  $203.5^{\circ}$  with decomposition. Soluble in 6 parts of cold and 2.5 parts of boiling water. Insoluble in alcohol and in ether. Notes on birotation are given. Aqueous solution reduces hot Fehling's solution. Lactose dissolved in twice its weight of nitric acid (*sp. gr.* 1.4) and heated, oxidises, and the products of oxidation, when mixed with water, separate as a mixture of a crystalline and amorphous powder. Lactose should be completely soluble in water, and leave no ash.

The last edition (1926) of the United States Pharmacopœia has the following:—1 grm. is soluble in 4.9 c.c. of water at  $25^{\circ}$  C., also in 2.6 c.c. of boiling water.

**Tests for Identity.**—The specific rotation ( $\alpha$ )<sub>D</sub> of lactose, determined at  $25^{\circ}$  C. in an aqueous solution containing in each 100 c.c. 10 grm. of lactose, previously dried to constant weight at  $80^{\circ}$ , and using a 200 mm. tube, is not less than  $+52.2^{\circ}$  and not more than  $+52.5^{\circ}$ .

The solution must be allowed to stand over night before observing the rotation.

Add an equal volume of sodium hydroxide (T.S.) to 5 c.c. of a hot, saturated, aqueous solution of lactose, and gently warm the mixture: the liquid turns yellow and finally brownish-red. On the subsequent addition of a few drops of cupric sulphate (T.S.), a precipitate of cuprous oxide forms.

*Tests for Purity.*—An aqueous solution of Lactose (1 in 20) is neutral to litmus paper.

Ash: not more than 0.1%.

A solution of 3 gramm. of lactose in 10 c.c. of boiling distilled water is clear, colourless, and odourless.

Add 20 c.c. of 70% (by volume) alcohol to 2 gramm. of finely powdered Lactose, shake the mixture frequently during half an hour at 15°, and filter: a 10 c.c. portion of the filtrate remains clear after mixing with an equal volume of dehydrated alcohol (dextrin), and this liquid upon evaporation on a water bath leaves not more than 0.03 gramm. of residue (sucrose or glucose).

An aqueous solution of lactose (1 in 20) meets the requirements of the test for heavy metals.

Boil 1 gramm. of powdered Lactose with 50 c.c. of distilled water, cool, and add a drop of iodine T.S.: no blue colour is produced (starch).

The German Pharmacopœia II states that if 0.2 gramm. of the milk sugar is spread on 1 gramm. of conc. sulphuric acid no colour, or at least only a very slight red, should appear—on no account should it go black (*v. infra*).

Similar tests are recorded in other Pharmacopœias.

*The Resorcinol Test.*—This is another useful test, which has been given under many forms. The following is one of the simplest methods of application: 1 gramm. of milk sugar is dissolved in 10 c.c. of water and mixed with 0.1 gramm. of resorcinol and 1 c.c. of conc. hydrochloric acid, and the mixture boiled for 5 minutes, within which time no red colour should appear. Pinoff has given the following modification: 0.05 gramm. of the sugar is mixed with 5 c.c. of alcoholic sulphuric acid (750 c.c. 96% alcohol, 200 c.c. of conc. sulphuric acid) together with 5 c.c. of alcohol—and 5 c.c. of an alcoholic solution of resorcinol, and the whole warmed to 95 to 98°. Sucrose, raffinose, fructose, and sorbose give within 1 minute a deep red colour, but 30 minutes' heating is necessary to give a colour with lactose, dextrose and maltose. The writers are of the opinion that the resorcinol test is not always reliable.

Anselmino has suggested fermentation of a 10% solution with a small quantity of freshly washed beer yeast at 20 to 30° for 2 days, within which time no gas should develop, if sucrose or invert sugar is absent.



The Pharm. Germanica IV, 1st Jan., 1901, gives the following test for examining milk sugar: 15 grm. of powdered milk sugar are mixed with 50 c.c. of *spiritus dilutus*, and allowed to stand for  $\frac{1}{2}$  hour, with repeated shaking. The liquid is then filtered and the filtrate should neither give any turbidity when mixed with an equal volume of absolute alcohol, nor should 10 c.c., when evaporated on the water-bath, give a residue of more than 0.04 grm. The test to be carried out at 15°.

*Spiritus dilutus* is defined as having a sp. gr. of 0.892–0.896, and contains 68 to 69 parts of alcohol by volume or 60 to 61% by weight.

The 5th edition of the German Pharmacopœia returns to the sulphuric acid test for the presence of sucrose. The method there prescribed being as follows: 0.5 grm. of finely powdered milk sugar is mixed with 10 c.c. of conc. sulphuric acid (1.836 to 1.841) in a test-tube previously washed with the acid, when the mixture should only be slightly yellowish within 1 hour, and on no account brown.

The 6th edition omits the test: it provides that a hot aqueous 1:1 solution of the sugar should be clear and at the most slightly yellow. The saturated aqueous solution should scarcely affect litmus paper and should not show any colouration with hydrogen sulphide after the addition of ammonia. There should not be more than 0.25% of ash.

Burr and Berberich, working under the conditions of the sulphuric acid test of the 5th edition, find the following times and colours for various percentages of sucrose.

Sucrose, %	5 minutes	10 minutes	15 minutes
1	Slightly yellow.	.....	Dark yellow.
2	Yellow	Dark yellow.	Brown.
3	Yellow.	Dark yellow.	Brown.
4	Yellow.	Dark yellow.	Deep brown.
5	Brownish-yellow.	Brownish-black.	Blackish-brown.

## B. (d) BY-PRODUCTS OF MILK SUGAR MANUFACTURE

During the clarification of the whey a considerable quantity of protein matter is obtained as a by-product, and is used either as a manure, or fodder, or for making into cheese, or for incorporating

with bread. The composition varies considerably, but the following analyses of the by-product (Ziger) from cow's milk whey have been published respectively by Klenze-enging and Fleischmann.

	%	%
Water.....	68.47	69.02
Total solids.....	31.53	30.98
Fat.....	5.22	0.47
Protein.....	18.72	13.18
Lactose.....	3.97	12.39
Ash.....	3.62	4.94

The analysis of the ash of some pressed Ziger was as follows:

CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>4</sub>	Cl	Alkali
22.2%	3.6%	1.5%	25.52%	0.58%	17.77%	balance%

**Milk Sugar Molasses.**—The following mean analyses of the uncrySTALLISABLE residues are given by Burr and Berberich: water, 72.67; total solids, 27.33; total ash, 6.03; water soluble ash, 3.98; water insoluble ash, 2.05; fat (Gottlieb), 0.09; nitrogen, 0.63; acidity (as lactic acid), 1.47; reducing substances (calculated as lactose), 22.29.

**ANALYSIS OF MILK SUGAR.**—Beyond the rough tests for other sugars described above, various estimations are sometimes required in judging the purity of milk sugar.

**Moisture.**—This is usually estimated by drying 5 grm. to constant weight in the water-oven, preferably in a wide flat weighing bottle. As it must be borne in mind that commercial milk sugar is usually a mixture of monohydrate and anhydrous milk sugar, the writers prefer to estimate the actual moisture in a vacuum desiccator; the combined water is then estimated on the same sample by heating at 140° to constant weight.

**Fat.**—This may be estimated on 2.5 grm. by the Gottlieb method.

**Nitrogen.**—5 grm. are treated by the Kjeldahl-Gunning method, or if great accuracy is required, it is preferable to dissolve a larger quantity in water, precipitate the protein with phosphotungstic acid and to estimate the nitrogen in the precipitate after filtration.

**Acidity.**—10 grm. dissolved in 50 c.c. of water are titrated with N/10 sodium hydroxide to phenolphthalein. Each c.c. equals 0.009 grm. of lactic acid.

The estimation of acidity is important, as any excess may cause trouble when used for modified milks.

**Sugar.** (1) *By the Polarimeter.*—The writers prefer to make a 5% solution of the sample, adding 7 c.c. of Wiley's dilute acid mercuric nitrate solution before making up to the mark. A direct reading is made at once, and the remainder of the solution counterpoised, then heated for 7 minutes in boiling water, cooled, readjusted to the original weight and again polarised. The two readings should be practically identical. This procedure allows of the estimation of any appreciable quantity of cane sugar which may be present (see condensed milk), while the production of turbidity or an actual precipitate gives an indication of protein present.  $1^{\circ}$  Ventzke = 0.31213 grm. of anhydrous lactose dissolved in 100 Möhr c.c. (see also page 147).

(2) *By Fehling's method* in the usual manner, and in the case of the volumetric method, the solution should be standardised against pure lactose.

**Ash.**—The ash is best estimated in the following manner: 10 grm. are incinerated at a low temperature until completely carbonised, the char then extracted with water, the filtrate evaporated and very gently ignited, the result being the *soluble* ash. The extracted char is ignited till completely incinerated, giving the *insoluble* ash. The sum of the two equals the *total* ash.

**Analysis of the Ash.**—It is often of importance to know the proportion of the constituents of the ash, which may be estimated by the well-known methods of mineral analysis. As this involves the incineration of very large quantities of milk sugar, the writers desire to draw attention to the value of the methods described by Thresh (*The Examination of Water and Water Supplies*, 1904) for the examination of the saline constituents of water, for this purpose.

Fifty grm. of the sugar are ignited, and the char after extraction with not more than 40 c.c. of water, is burnt completely, and its ash dissolved in a few drops of hot dilute hydrochloric acid, and added to the solution of the soluble ash. The whole solution, which should not exceed 50 c.c., is heated to boiling and 2 c.c. of a saturated solution of ammonium oxalate added and then dilute ammonia till just alkaline to litmus; the solution is then made distinctly acid with acetic acid, and the whole allowed to stand covered on the water-bath for about an hour. The calcium oxalate is then filtered off on a

small hardened filter, and the flask and precipitate washed free from chlorides—the washings should not exceed 50 c.c. The calcium oxalate is washed off the filter back into the original flask, the filter being finally washed with 10 c.c. of boiling 10% sulphuric acid. The solution of the oxalate is then heated to 50° and titrated with permanganate solution (3.95 grm. per litre. 1 c.c. permanganate solution equals 0.0025 grm. calcium).

The filtrate from the calcium oxalate is cooled and made up to 55 c.c. To 4 c.c. 2 to 3 drops are added of ammonium phosphate solution (made by dissolving 10 grm. of ammonium phosphate in 100 c.c. of water and adding ammonia (0.88) until a permanent turbidity results; the solution is then filtered). If a heavy precipitate is produced on shaking or scratching the sides of the test-tube, 25 c.c. or less of the solution may be used, but if only a slight turbidity is formed 50 c.c. will be required. The necessary amount is placed in a 50 c.c. Nessler tube, and made up to the mark if required. 1 c.c. of the ammoniacal solution of ammonium phosphate is added and the whole *vigorously* stirred for 2 minutes with a flat disc plunger of nearly the same diameter as the tube.

A graduated 50 c.c. Nessler glass is then placed in a good diffused light, on a disc of white cardboard thickly covered with black dots. The test solution is slowly poured in until the dots can be no longer distinguished, and the number of cubic centimetres required is noted; the process is repeated 2 or 3 times, the mean of the readings being taken. A test is then carried out in an exactly similar manner with a solution of magnesium chloride containing 0.00025 of magnesium per c.c. (made by dissolving 0.025 grm. of pure magnesium wire in dilute hydrochloric acid and diluting to 1,000 c.c.). A sufficient quantity of this is diluted to 50 c.c. and treated exactly as the test solution, in order to give a turbidity which will obscure the dots in practically the same amount as the test solution. By comparison, the amount of magnesium in the test solution is easily ascertained. The method is exceedingly accurate and more reliable for these small quantities than the usual gravimetric method.

**Sulphates** are quite easily estimated in a similar manner. About 20 to 25 grm. of the sample are ashed as above described; the ash is dissolved in a small quantity of hydrochloric acid and the solution made up to 100 c.c. To 50 c.c. of this solution in a Nessler tube are added 1 c.c. of dilute hydrochloric acid (1 in 3) and 0.5

c.c. of an acid solution of barium chloride (made by dissolving 10 grm. of barium chloride in 80 c.c. of water and adding 20 c.c. of hydrochloric acid) stirred vigorously and the turbidity point ascertained, as in the case of magnesium. This is then compared with that of a standard solution of sulphuric acid containing 0.0032 grm. of  $\text{SO}_4$  (made by diluting 100 c.c.  $N/10$  sulphuric acid to 150 c.c.). The test must be carried out rapidly, owing to the tendency of barium sulphate to settle out. If the amount of sulphate is at all excessive the sulphate is more accurately estimated after the above approximate estimation, in the following way: the reserved 50 c.c. is boiled and a standard solution of barium chloride (containing 5.071 grm. of pure cryst. barium chloride in 1,000 c.c. of water, of which solution 1 c.c. = 0.002 grm. of  $\text{SO}_4$ ), added in slight excess of that required to precipitate the whole of the  $\text{SO}_4$  as already estimated. The liquid is allowed to stand 10 minutes, and a dilute solution of ammonia (free from carbonate) added till the solution is faintly alkaline. It is again boiled and a standard solution of potassium chromate (containing 4.05 grm. per 1,000 c.c., which is equivalent to the barium chloride solution) added, 0.5 c.c. at a time, until the supernatant liquid is distinctly yellow. The whole is then filtered into a Nessler glass, made up to 50 c.c., and the colour matched by running the standard chromate solution into 50 c.c. of distilled water placed by the side. The excess of chromate over that required to precipitate the excess of barium chloride solution is thus found, and so the  $\text{SO}_4$  in the ash obtained.

**Phosphates** can be estimated on another portion of the ash. The ash is dissolved in dilute nitric acid and evaporated to dryness (*in a porcelain dish*) heated to  $120^\circ$  for 15 minutes and dissolved in 20 c.c. of distilled water, containing 2% of nitric acid. 5 c.c. of ammonium molybdate solution are placed in a test-tube and heated to  $60^\circ$  and 2 to 3 c.c. of the test liquid added, and the whole allowed to stand for 15 minutes in a warm place—this preliminary test will show how much of the test solution must be used to obtain a good yellow colour without actual precipitation. The colour is then matched by that given by a standard solution of sodium phosphate, containing 0.0001 grm. of  $\text{PO}_4$  per c.c.

The writer has the utmost confidence in the above methods, and in cases in which estimation of the above constituents have to be

made for control purposes, the saving of time over the ordinary gravimetric methods is enormous.

### SECTION C. CONDENSED MILK

This form of milk product has been brought to a great state of perfection and has in consequence acquired a considerable and well-deserved popularity. It appears on the market in two forms: (1) Sweetened condensed milk, whole and skimmed, and (2) unsweetened condensed milk, whole and skimmed. The chief difference between the two varieties is the presence of a considerable percentage of cane sugar in the former, the addition of which obviates the rigorous sterilisation to which the latter has to be subjected.

The sugar added has not probably in itself any preservative action, but the high density so produced causes plasmolysis of the organisms present and so prevents their growth. As the temperature of the vacuum pan has little sterilising effect, organisms would develop in the unsweetened variety, if rigorous sterilisation were not carried out. It is to the growth of organisms of a sporogeneous type that the bitterness and protein degradation, so characteristic of some of the earlier unsweetened milks, must be attributed.

The process of treatment of the milk is practically the same in either case. The following is the procedure at one of the largest and best known makers of condensed milks: The fresh milk is brought each day to the factory by farmers in the vicinity and is run into large tanks after passing through strainers (centrifugal cleaners are often used). From the tanks the milk runs through a pasteuriser, both for the purpose of heating the milk sufficiently to dissolve the sugar when added and also to obviate all risk from pathogenic organisms.

The hot milk is run from the pasteurisers into tanks in which the fine cane sugar is mixed with the milk by means of stirrers. The sweetened milk is run direct into the vacuum pans, in which it is evaporated to the desired concentration, the process taking about 3 hours. The condensed milk is run off into drums placed in cold water and fitted with a stirring device driven from beneath, the cooled milk being then filled into the tins.

In the case of unsweetened condensed milk the milk is run from the pasteurisers after suitable cooling to the vacuum pans. After condensation the warm milk is homogenised (see page 151), as this pro-

cess by its effect on the cream imparts a stability to the product which enables it to stand storage and transit in a way otherwise impossible. The homogenised condensed milk is filled into tins which are then packed into large rotating sterilisers and subjected to a temperature of about 105°.

The strictest cleanliness has to be observed in every stage of the process, and there are many technical details involved in the production of a high-class product especially in regard to the temperatures employed. In a high-class condensed milk of either variety, there is not the least need for the addition of any preservative, such as was the common practice in earlier days of milk condensation.

The writers find the sp. gr. of sweetened condensed milk in the most important brands to be usually 1.32 *circa*.

The density is estimated in a 10% solution, and from this the density of the original condensed milk is calculated by means of the following formula:

$$\text{Sp. gr. cond.} = \frac{1}{11 - 10 \text{ Sp. gr. diluted milk.}}$$

In the case of unsweetened condensed milk, the extent to which the concentration has been carried may be judged from the proportion of non-fatty solids. The percentage of ash affords an independent criterion, but this is liable to be vitiated if mineral preservatives have been added. Further, a deposition of certain salts is liable to occur during the evaporation of the milk, and this circumstance tends to diminish the proportion of ash in the finished product.

As the fat of cows' milk is usually in excess of the protein, it will be at least as high as the protein in the condensed preparation, provided that none of it has been removed. There is considerable inducement to remove part of the milk fat prior to concentration, as a portion is liable to separate from very rich milk, and this difficulty has only been overcome of late years by homogenisation. Such removal of part of the milk fat prior to concentration is not now allowed in England.

Besides preservatives, certain materials such as glucose syrup, wheat flour and starch have been sometimes added in low-grade products in order to create thickness and to prevent solidification of the condensed product. Saccharate of lime has also been used for this purpose.

The following requirements were suggested by a Departmental Committee (England) in their Report of 1901:

- (1) The fat must not be less than 10%, and must be true butter fat.
- (2) The protein (estimated by multiplying the nitrogen by 6.39) must not exceed the figure obtained for the fat.
- (3) The sample must be free from preservatives, starch, and all other foreign matters.

These requirements have been superseded by the Public Health (Condensed Milk) Regulations, 1923, made by the Minister of Health and the Public Health (Condensed Milk) Amendment Regulations, 1927 (Statutory Rules and Orders 1923, No. 509 and 1927, No. 1092).

These regulations define four forms of condensed milk and control its composition so far as the proportions of milk fat and milk solids are concerned. They also require the container to be labelled clearly with the correct description, the equivalent of the contents in pints of fresh milk and, in the case of condensed skimmed milk and condensed machine-skimmed milk, with a label stating that the product is "*unfit for babies.*" No preservative may be used in the preparation of condensed milk.

The requirements for the percentage of milk fat and milk solids are as follows:

Description of condensed milk	Percentage of milk fat	Percentage of all milk solids including fat
1. Full cream unsweetened.....	9 0	31.0
2. Full cream sweetened.....	9 0	31.0
3. Skimmed unsweetened.....		20.0
4. Skimmed sweetened.....		26 0

The corresponding standards of the U. S. Department of Agriculture, which have been adopted in whole or part by many of the States Authorities, are as shown in table on p. 134.

Canada prescribes a minimum of 26% of milk solids and 7.2% of fat for evaporated milk, and 28% of milk solids and 7.7% of fat for sweetened condensed milk.

For the purpose of calculating the equivalent of the contents of the container in terms of fresh milk, the English Regulations define



Description of condensed milk	Percentage of milk fat	Percentage of total milk solids including fat
1. Condensed, evaporated or concentrated . . .	7.8	25.5
2. Sweetened condensed, evaporated or concentrated . . . . .	8.0	28.0
3. Condensed, evaporated or concentrated skimmed . . . . .		20.0
4. Sweetened condensed, evaporated or concentrated skimmed . . . . .		24.0

milk as "milk which contains not less than 12.4% of milk solids including not less than 3.6% of milk fat," and skimmed milk as "milk which contains not less than 9% of milk solids other than milk fat."

Tables have been prepared for assisting in the calculation of the "equivalent pints" from the net weight of the contents of a can and the percentage of milk fat and milk solids by Hinks (*Analyst*, 1923, 48, 596), Essery (*Analyst*, 1924, 49, 178), and Liverseege (*Analyst*, 1924, 49, 276).

The following analyses of unsweetened condensed milk are by Hunziker and Spitzer (1909):

Brand	Total solids, %	Fat, %	Protein, %	Sugar, %	Ash, %
Goldmilk . . . . .	29.25	9.42	8.44	9.75	1.54
Columbine . . . . .	24.63	7.45	7.41	8.56	1.36
Every Day . . . . .	26.20	8.07	7.54	9.10	1.47
Star . . . . .	29.04	8.35	7.86	10.37	1.62
Morning Glory . . . . .	31.08	10.48	8.26	10.47	1.67
Carnation . . . . .	23.81	8.05	6.49	7.55	1.24
Beauty . . . . .	23.38	8.47	8.39	9.94	1.56
Van Camp's . . . . .	27.89	8.69	7.52	9.66	1.54
Wilson's . . . . .	25.23	8.70	6.53	8.68	1.37
Monarch . . . . .	26.70	8.09	6.77	10.35	1.44
Diadem . . . . .	24.96	8.16	7.06	7.92	1.33
Reindeer . . . . .	26.66	8.08	6.88	10.21	1.45
Dundee . . . . .	27.04	8.73	7.21	9.36	1.48

The following unpublished analyses, which have been kindly supplied to us by Mr. T. Macara, are typical of the condensed milks of the period immediately prior to 1914.

The analyses in the first three sections show the variations which may take place in the product of one maker:

Source	Sample	Fat, %	Lactose, %	Protein, %	Ash, %	Sucrose, %	Water, %	Total milk solids, %
Maker A Holland.	1	8.00	14.21	8.80	2.20	41.77	25.02	33.20
	2	7.52	13.30	8.87	2.06	43.00	25.25	31.75
	3	11.00	13.29	8.74	2.08	39.65	25.24	35.11
	4	12.58	13.20	9.00	1.90	38.00	25.32	36.68
Maker B Holland.	1	9.94	13.20	9.57	2.00	39.30	25.99	34.71
	2	8.94	14.24	9.02	2.00	41.20	24.60	34.20
	3	9.64	14.00	8.87	1.90	40.76	24.83	34.41
	4	9.30	14.40	8.93	1.93	41.95	23.49	34.56
Maker C Holland.	5	9.72	13.95	8.30	1.89	40.97	25.17	33.86
	6	10.50	14.20	8.04	1.90	40.20	25.16	34.64
	1	9.40	13.86	9.20	2.10	40.86	24.58	34.56
	2	9.56	13.00	8.68	2.00	40.20	26.56	32.24
Various makes.	3	9.50	13.54	8.22	2.00	41.10	25.64	30.26
	1	9.7	13.40	9.00	1.90	41.60	24.40	34.00
	2	8.7	13.30	9.20	1.80	42.30	24.70	33.00
	3	7.70	10.00	8.12	1.93	38.95	30.30	30.75
Machine skimmed.	4	8.66	11.00	7.41	1.65	43.50	27.78	28.72
	5	11.26	14.50	8.49	1.85	39.80	24.10	30.10
	1	0.34	13.66	9.04	2.20	44.90	29.26	23.64
	2	0.70	14.04	9.57	2.34	44.27	28.18	27.55
Various makes.	3	0.38	14.60	10.21	2.37	43.52	28.92	27.50
	4	0.34	13.75	8.31	2.04	41.96	33.60	24.44

None of these contained boron compounds.

All the above constituents were estimated practically as described in the text, except that the protein was precipitated with phosphotungstic acid, the filtered precipitate being treated by the Kjeldahl method, and the lactose was estimated by a Fehling's method. Total solids in all cases were estimated to check the calculated water. Macara is of the opinion that the ash is quite unreliable for determining the concentration. Of the 4 milks under Maker "A" Holland, the first 2 were condemned by him as made from partly skimmed milk, whereupon milks giving the analytical figures of 3 and 4 were received from the same maker.

The following are the limits of variation found in a large number of analyses collected by Dr. Coutts for a report to the Local Government Board (England), 1911.

	Full cream				Machine skimmed	
	Sweetened		Unsweetened		Sweetened	
	Lowest	Highest	Lowest	Highest	Lowest	Highest
Total solids, %	68.1	83.6	29.2	38.0	56.9	79.1
Fat, %	8.0	13.7	8.2	11.9	0.1	6.5 <sup>1</sup>
Protein, %	7.3	11.4	8.0	10.0	7.6	12.3
Ash, %	1.6	3.4 <sup>1</sup>	1.6	2.5	1.6	2.9
Lactose, %	11.6	17.6	11.1	16.0	10.9	17.0
Cane sugar, %	36.1	44.6	.....	.....	30.4	52.6

<sup>1</sup> Probably contains sodium hydrogen carbonate or boric acid.

<sup>2</sup> Only partially skimmed.

In the period between 1918 and 1923, when the English regulations came into force, the limits were somewhat narrower and the better class products generally complied with the compositions now required. The following tables show typical analyses of condensed milk, full cream and skimmed, sweetened and unsweetened, as now sold in England.

#### FULL CREAM, SWEETENED CONDENSED MILK

Fat.....	9.08	9.22	9.07	9.13	9.53	9.90
Protein.....	8.09	7.84	9.02	7.88	9.28	9.46
Lactose.....	12.78	12.90	13.72	13.20	12.91	12.82
Ash.....	1.96	1.73	1.95	1.93	2.06	2.05
Sucrose.....	42.42	41.03	41.70	42.10	40.40	41.00
Water.....	25.67	27.28	24.54	25.78	25.82	24.77
	100.00	100.00	100.00	100.00	100.00	100.00
Milk solids.....	31.91	31.69	33.73	32.12	33.78	34.23

#### FULL CREAM, UNSWEETENED CONDENSED MILK

Fat.....	9.12	9.26	9.34	9.01	9.65
Protein.....	8.38	8.40	8.32	8.62	8.70
Lactose.....	12.22	12.79	11.91	11.85	11.95
Ash.....	1.96	2.00	1.94	2.04	1.90
Water.....	68.32	67.55	68.40	68.48	67.80
	100.00	100.00	100.00	100.00	100.00
Milk solids.....	31.68	32.45	31.51	31.52	32.10

#### SKIMMED, SWEETENED CONDENSED MILK

Fat.....	0.46	0.24	0.39	0.19	0.62	0.20
Protein.....	9.75	10.79	10.83	11.10	10.33	11.80
Lactose.....	13.70	13.53	14.33	14.38	13.17	14.60
Ash.....	2.96	2.12	2.15	2.28	1.98	2.35
Sucrose.....	44.76	47.67	45.90	45.75	47.35	46.25
Water.....	28.37	25.65	26.40	26.30	26.55	24.80
	100.00	100.00	100.00	100.00	100.00	100.00
Milk solids.....	26.87	26.68	27.70	27.95	26.10	28.95

#### Analysis of Condensed Milk

The *Milk Products Sub-Committee of the Standing Committee on Uniformity of Analytical Methods of the Society of Public Analysts* has put forward methods for the determination of total-solids, fat

and sugar in condensed milks (*Analyst*, 1927, 52, 402 and 1930, 55, 111).

This Committee recognises that the sampling of such a product as condensed milk is a matter of considerable importance. It could not deal adequately with this subject until satisfactory methods of analysis had been selected, but with reference to the preparation of a sample submitted for laboratory examination it had the following observation to make: The most efficient mixing was obtained by hand, using a spoon with an up and down rotatory movement, in such a way that the top layers and the contents of the lower corners of the containing vessel were moved and mixed, care being taken that any separated crystals in the original sample should first be ground and incorporated in the bulk. It is important that frothing or the formation of air bubbles should be avoided.

Because of the difficulty of accurately measuring volumes of the diluted condensed milk and the danger of the separation of fat from the liquid, the principle of taking weighed quantities of the sample sufficient for each determination was adopted in preference to taking aliquot parts by volume of a liquid prepared by diluting a larger quantity of the sample with water. The relatively small weighed quantities that must be taken for analysis emphasise the importance of complete mixing of the sample.

**Total Solids.**—The Society of Public Analysts' Committee recommends the following method of determination:

*Preparation of the Support.*—Select for use sand which passes a 30-mesh and is retained by a 90 mesh sieve. Heat a convenient quantity of this sand with strong hydrochloric acid to remove oxide of iron, etc.; decant; repeat the digestion till the acid liquor is nearly colourless; wash, once with dilute hydrochloric acid, and then thoroughly with distilled water; dry, and ignite.

The sand thus prepared should be tested for suitability as follows: Dry a portion at 98°–100° C. and weigh; moisten with distilled water and subsequently dry again at 98°–100° C. There should be no difference between the two weights.

*Dishes.*—These should be of metal (aluminium or nickel is suitable), with readily removable but close fitting lids; a suitable size is of diameter about 3 inches and depth about 1 inch.

**Procedure.**—(1) *Sweetened Condensed Milk.*—Place about 25 grms. of the prepared sand and a short glass stirring rod in the dish and

dry to constant weight in an oven at  $98^{\circ}$ – $100^{\circ}$  C., the lid being removed whilst drying and replaced before removing the dish from the oven. Allow the dish to remain for 45 minutes in the desiccator before weighing.

Tilt the sand to one side of the dish; place on the clear space about 1.5 grms. of the well-mixed sample and weigh rapidly. Add 5 ml. of water to the milk and mix these; then mix the diluted milk thoroughly with the sand by means of the rod.

Place the dish on the rapidly boiling water bath for 20 minutes, carefully stirring during the earlier period. Transfer the dish, with rod and cover, to a well-ventilated oven at  $98^{\circ}$ – $100^{\circ}$  C., as recorded by a thermometer in the air immediately above the dish. After  $1\frac{1}{2}$  hours, cover the dish and place in the desiccator for 45 minutes; weigh; return the dish to the oven, and heat for one hour with lid removed; remove and weigh as before; repeat this process until the loss of weight between successive weighings does not exceed 0.0005 gm.

**Fat.**—There is but a limited choice of methods for this determination. Rapid methods, such as the Gerber, the Babcock, and the Leffman-Beam, are capable of giving approximately correct results, but are not suited for determination of fat, with the desired degree of accuracy. The Werner-Schmid process gives good results with unsweetened milks, but is not desirable for sweetened milks. The ultimate choice of a method applicable to both sweetened and unsweetened milks lay between a coagulation method and the Röse-Gottlieb method. Coagulation by copper sulphate and by dialysed iron were investigated; their advantage lies in the fact that relatively large quantities of the sample can be taken for analysis: their disadvantage in the necessity for the adoption of some such treatment of the coagulum as that of Werner-Schmid. These methods are capable of giving accurate results, but the Committee considered that the Röse-Gottlieb process, with the modifications detailed hereafter, is preferable, and accordingly recommends its use.

In the process recommended it has been found that very strict adherence to details is essential.

The Committee recommends the use of the following modification of the Röse-Gottlieb method:

*Reagents.*—Concentrated ammonia solution, nominal 0.880.

Alcohol or industrial methylated spirit, 95% by volume.

Ether (methylated), sp. gr., 0.720.

Petroleum spirit, boiling between 40° and 60°.

These reagents should leave no appreciable residue on evaporation.

*Procedure.*—Transfer to a suitable apparatus from 2 to 2.5 gm., accurately weighed, of the well-mixed sample; add 8 ml. of warm water and mix well; cool; add 1 ml. of concentrated ammonia solution, mix, add 10 ml. of alcohol and again mix. Add 25 ml. of ether and shake vigorously for 1 minute; add 25 ml. of petroleum spirit and again shake vigorously for 30 seconds. Allow the liquids to stand for not less than half an hour, until the ethereal layer is perfectly clear, or centrifuge at a low speed. Transfer the ethereal layer to a suitable flask. To the milk residue add 5 ml. of ether, and transfer without further shaking; repeat this operation in the same manner with a further 5 ml. of ether. Add 0.5 ml. of alcohol, and repeat the extraction with 25 ml. of ether and 25 ml. of petroleum spirit, as before, shaking vigorously for one minute after the addition of the ether and for 30 seconds after the addition of the petroleum spirit. As before, allow the ethereal layer to separate completely and transfer to the flask. Repeat the extraction once more with alcohol, ether and petroleum spirit in the same manner.

Cautiously distil the solvents from the flask and dry the residual fat at 98°–100° C. to constant weight, taking the ordinary precautions to remove all traces of volatile solvent.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation. Finally dry the flask at 98°–100°. The difference in weights before and after the petroleum spirit extractions is the weight of fat contained in the quantity of condensed milk taken.

Make a blank determination, using the specified quantities of reagents, and distilled water in place of the milk, and deduct the figure found, if any, from the weight of fat obtained.

**Ash.**—2 gm. of the milk are weighed into a platinum dish, diluted with about 8 c.c. of water and the liquid is carefully mixed. The solution is evaporated as far as possible on a boiling water bath and then carefully burnt at a low red heat in a muffle or over a burner, the incineration in the latter case being materially hastened by placing another platinum dish partially over the original dish. If special accuracy in the ash figure is required, a larger quantity of the sample should be taken.

**Protein.**—1 gram of the original sample is placed in a digestion flask and the water rapidly boiled off and the nitrogen estimated by the Kjeldahl-Gunning method.

If only an approximate estimation of the protein is required, the aldehyde figure of Steinegger may be employed. For this purpose 2 grams of the sample are titrated with  $N/10$  sodium hydroxide to the neutral point using phenolphthalein; to the neutralised solution 5 c.c. of 40% formaldehyde solution (previously neutralised) are added and the titration continued till a second neutral point is obtained. The difference between these 2 titrations is expressed as c.c. of  $N$  sodium hydroxide solution per 1,000 c.c. of milk, and is the aldehyde figure. The percentage of protein in the dilution employed is calculated from the following formula:

$$\text{Protein} = \text{Aldehyde figure} \times 0.225$$

The above method is useful for correcting for the volume of protein when estimating the amount of sugars. Richmond uses strontium hydroxide.

**Sugars.**—The English Condensed Milk Regulations, referred to above, require sweetened condensed milks to contain specified proportions of milk solids; and since it is now considered that the proportion of total milk solids is best obtained by subtracting the proportion of sucrose present from that of total solids, much attention has been directed recently to reviewing and improving the methods available for determining sucrose in milk.

The Society of Public Analysts' Committee (*Analyst*, 1930, 55, 111) has reviewed these methods and grouped them under three headings:

1. Copper Reduction Methods.
2. Iodimetric and Chloramine-T methods.
3. Polarimetric methods.

The copper reduction methods were investigated at considerable length and were finally abandoned for the determination of sucrose, because it was found that too many factors influenced the uniformity of results and that the desired accuracy of within  $\pm 0.5\%$  of the sucrose present could not be consistently obtained.

So far as Iodimetric and Chloramine-T methods are concerned, the Committee found the Chloramine-T process of Hinton and Macara to be superior to the direct iodimetric process. The Hinton

and Macara method is regarded as promising, but the Committee found that in its present form it did not yield the desired degree of accuracy in the determination of sucrose.

They were thus led to the conclusion that, whatever process might be adopted, the application of a polarimetric method would almost certainly be involved, especially in view of the possible presence of invert sugar in certain cases.

In arriving at this conclusion they have agreed with the findings of the writers, as published in the last edition of this work.

The Committee has made a detailed investigation of the following factors necessarily involved in any process based, as were all the polarimetric methods examined, upon the Clerget process:

- (a) Clarification.
- (b) Muta-rotation.
- (c) Effect of non-sugars: "neutral" and "acid" polarisation.
- (d) The inversion.
- (e) The inversion divisor factor.
- (f) The correction for the volume of the precipitate produced in the clarification.

After paying particular attention to the factor (f)—which is the most difficult phase of all polarimetric processes—they have evolved the following method:

## DETERMINATION OF SUCROSE

### The Society of Public Analysts' Method for Determination in Sweetened Condensed Milk

*Reagents.*—Zinc acetate solution: 21.9 grm. of crystallised zinc acetate,  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$  and 3 ml. of glacial acetic acid, in water, made up to 100 ml.

Potassium ferrocyanide solution: 10.6 grm. of crystallised potassium ferrocyanide in water made up to 100 ml.

Hydrochloric acid solution = 6.34 times normal.

Concentrated ammonia solution: nominal 0.880.

Dilute ammonia solution: 10 ml. of concentrated ammonia solution diluted with water to 100 ml.

Dilute acetic solution approximately equivalent to the dilute ammonia solution.



*Apparatus.*—The instrument used for measuring the optical rotation may be either a polarimeter or a saccharimeter, using, for the polarimeter, sodium light, or the green line of the mercury spectrum separated by means of a prism or by the use of a special Wratten screen No. 77a, and for the saccharimeter white light from an incandescent electric lamp after passing through 15 mm. of a 6% solution of potassium dichromate.

Tubes, of not less than 2 dm., exactly calibrated for length.

Flasks and pipettes accurately calibrated in millimeters.

A standardised thermometer, reading to 0.1° C.

*Preparation of the Sample.*—Mix the sample in the manner prescribed above.

*Procedure.*—Transfer to a 100 ml. beaker an accurately weighed quantity, approximately 40 grms., of the well-mixed sample; add 50 ml. of hot distilled water (80°–90°), mix, transfer to a 200 ml. measuring flask, washing in with successive quantities of distilled water at 60°, until the total volume is from 120 to 150 ml. Mix, cool to air temperature, and then add 5 ml. of the dilute ammonia solution. Again mix, and allow to stand for 15 minutes. Add a sufficient quantity of the dilute acetic acid solution to neutralise the ammonia added (the exact equivalent is determined beforehand by titration), and again mix. Add, with gentle mixing, 12.5 ml. of zinc acetate solution and mix, followed in the same manner by 12.5 ml. of potassium ferrocyanide solution. Bring the contents of the flask to 20° C. and add distilled water at 20° C. up to the 200 ml. mark.

Up to this stage all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles, and, with the same object in view, all mixings should be made by rotation of the flask rather than by shaking. If bubbles are found to be present before completion of dilution to 200 ml., their removal can be assisted by temporary attachment of the flask to a vacuum pump, and rotation of the flask.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes and then filter through a dry filter paper, rejecting the first 25 ml. of filtrate.

*Direct Polarisation.*—Determine the rotation of the filtrate at 20.0°.

*Inversion.*—Pipette 40 ml. of the filtrate obtained as above into a 50 ml. flask; add 6 ml. of 6.34 normal hydrochloric acid.

Immerse for 12 minutes the entire bulb of the flask in a water-bath maintained at 60°, mixing by rotatory movement during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, dilute to 50 ml. at 20° C. with distilled water, mix and allow to stand for one hour.

*Invert Polarisation.*—Determine the rotation at 20.0° C.

Calculation:

W = Weight of sample taken, in grms.

F = Percentage of fat in the sample.

P = Percentage of protein ( $N \times 6.38$ ) in the sample.

V = Volume to which the sample is diluted before filtration.

v = correction in ml. for volume of precipitate produced during clarification.

D = observed direct polarimeter reading.

I = observed invert polarimeter reading.

l = length in dm. of polarimeter tube.

Q = Inversion divisor factor.

Then

$$v = \frac{W}{100} \left[ (F \times 1.08) + (P \times 1.55) \right]$$

and the percentage of sucrose in the sample

$$= \frac{D - \left(\frac{5}{4} \times I\right)}{Q} \times \frac{V - v}{V} \times \frac{V}{l \times W}$$

*The Inversion Divisor Factor Q.*—The value of Q varies with the source of light, the type of polarimeter and the temperature used. Under the conditions of this method at 20° C., the appropriate values are, for:

Sodium light	0.8825
Mercury green line (prism or special Wratten screen No. 77a)	1.0392
International Sugar Scale (j) light	2.549

If the concentration or temperature vary from those described in the method of determination, a correction must be applied to the above factors; the value of Q is then found from the equations:

For sodium light

$$Q = 0.8825 + 0.0006(C - 9) - 0.0033(T - 20)$$

For mercury green line (prism or special Wratten screen No. 77a)

$$Q = 1.0392 + 0.0007(C - 9) - 0.0039(T - 20)$$

For International Sugar Scale (j) light

$$Q = 2.5490 + 0.0017(C - 9) - 0.0095(T - 20)$$

In these equations, C is the percentage of total sugars in the invert solution as polarised, and T the temperature at which the invert polarisation is measured.

### Monier-Williams' Methods

The main difficulty to be overcome in evolving polarimetric or other methods for the determination of sugars in condensed milks is the accurate determination of the volume of the precipitate obtained in preparing the milk serum. Monier-Williams has dealt with this difficulty in one method (*Analyst*, 1928, 53, 569) by determination of the proportion of water and total solids in the serum as well as in the milk, a knowledge of these factors enabling the problem to be solved with a fair degree of accuracy. The method, for details of which the original paper should be consulted, leads to results which are slightly high, owing to the fact that no account is taken of the state of hydration of the precipitant or the milk solids. Monier-Williams has therefore put forward a second method, in which a different principle is used. In this method the milk serum is prepared by the use of dialysed iron as precipitant; the precipitate is washed free from sugar by a special method and transferred completely to a solution containing a known weight of sucrose and lactose which is then made up to the same volume as the milk serum and precipitate occupied before filtration.

Comparison of the polarimetric readings obtained before and after inversion of the milk serum and sugar solution leads directly to the proportions of sucrose and lactose in the milk serum. The method is thus described (*Reports on Public Health and Medical Subjects*, No. 57, "The Determination of Sucrose, Lactose and Invert Sugar in Sweetened Condensed Milk"):

The protein precipitant employed in the method described below is the dialysed iron of the Pharmaceutical Codex containing about 5% of iron. This reagent was introduced as a protein precipitant by Michaelis and Rona (1908) and was used for the clari-

fication of milk in the determination of lactose by Oppenheim (1909). It has the advantage that the filtrate obtained is neutral, thus avoiding any danger of inversion of sucrose during the course of the analysis, also that any excess of iron used is precipitated and does not pass through into the filtrate. The clear filtrate shows no reaction for protein when tested with the usual protein reagents.

About 30 grm. of sweetened condensed milk, accurately weighed, are dissolved in warm water and the solution transferred to a 250 c.c. graduated flask, water being added until the volume is approximately 100 c.c. About 50 c.c. of dialysed iron are added from a burette very gradually with continued shaking of the flask contents. The flask is then placed in a boiling water-bath and gently agitated for three minutes. It is removed, cooled, and the contents made up to the mark with water. The heating serves to remove air bubbles which would interfere with the making up of the mixture to the mark, and also destroys any muta-rotation shown by lactose in condensed milk. Since the liquid is quite neutral, this heating causes no inversion of sucrose.

Several observers have pointed out the effect of heat in lowering the specific rotation of lactose (Richmond and Boseley, 1893; Jørgensen, 1924; Porcher and Bonis, 1918), but since a second determination is carried out using equivalent quantities of pure sucrose and lactose (see below) any slight error due to this cause is eliminated. On one occasion a sample was found in which a persistent froth was formed upon the surface of the liquid, which had to be broken down before attempting to make the mixture up to the mark. This was done by leading a jet of steam into the flask just above the surface of the froth, and gently agitating the contents of the flask.

The liquid, after being well mixed, is filtered through a plain dry filter in a funnel of about six inches diameter. The filtrate is allowed to stand for several hours (preferably overnight) and polarised (*Direct reading*, A).

Fifty c.c. of the filtrate are introduced into a 100 c.c. standard flask, 10 c.c. of 5 *N* HCl added and the sucrose inverted by immersion of the flask in a water-bath at 60° C. for 12 minutes, the flask being gently agitated for the first four minutes of immersion. The flask is withdrawn and the contents cooled rapidly, made up to the mark with water, allowed to stand several hours and polarised (*Invert reading* A).

The iron precipitate remaining on the filter has now to be washed free from sugar. If washed with hot water the filtration becomes progressively slower as sugar and soluble salts are removed, and it may take several hours before the washing is complete. By washing with a boiling 1% solution of common salt, and keeping the liquid on the filter in continuous agitation by means of a jet of steam the speed of filtration is very greatly increased, and complete freedom from sugar can usually be attained in one hour or less. Agitation by a jet of steam introduced below the surface of the liquid on the filter appears to be almost as important in securing quick filtration as is the presence of salt. It serves to keep the minute particles of precipitate from settling on the surface of the paper and blocking the pores, and at the same time maintains the temperature of the filtering liquid near the boiling point. The point at which all sugar has been washed out of the precipitate is ascertained by examining the filtrate in the polarimeter, when the zero reading should not show any alteration.

Ten grm. of pure sucrose and 3.5 grm. of pure lactose hydrate, both free from moisture and accurately weighed, are dissolved in a small quantity of warm water in the original 250 c.c. flask, which has previously been well washed out with hot water. The main part of the precipitate is detached from the filter by inversion of the funnel over a dish. The precipitate adhering to the paper is washed off by a jet of water and the whole precipitate rubbed up with water to a uniform thin cream and transferred to the 250 c.c. flask. The flask is heated in a boiling water-bath for three minutes as before, cooled, the contents made up to volume, filtered and polarised after standing several hours. (*Direct reading B.*) This polarisation should be carried out at as nearly as possible the same temperature as in the case of the direct polarisation A above. Fifty c.c. of the filtrate are inverted in the same way as already described, allowed to stand, and polarised at exactly the same temperature, as in the case of invert solution A, above. It is best so to arrange matters that both inversions and both invert polarisations can be carried out side by side under the same conditions. The actual temperature of polarisation is a matter of indifference provided it is the same in both cases. This obviates the necessity of adjusting the polarisation temperature exactly to 20°. If, however, the sample is suspected to contain much invert sugar, the temperature of polarisation should be as nearly as possible 20°.

The change on inversion in each case is obtained by multiplying the invert reading by 2 and subtracting it from the direct reading. The percentage of sucrose in the sample is then given by the following calculation:

$$\text{Sucrose per cent.} = \frac{S_2 \times P_1}{P_2} \times \frac{100}{W}$$

Where  $S_2$  = weight of sucrose taken for second polarisation.

$P_1$  = change on inversion of filtrate A

$P_2$  = change on inversion of filtrate B

$W$  = weight of sample taken.

**Lactose.**—The proportion of lactose may be calculated from the polarimetric readings obtained in the determination of sucrose in condensed milks; thus Monier-Williams gives the following formula for use in conjunction with the method described above:

$$\text{Lactose hydrate per cent.} = \frac{1619A}{WB} - 1.269S.$$

Where  $A$  is the direct rotation of filtrate A

$B$  is the direct rotation of filtrate B

$W$  is the weight of condensed milk taken

$S$  is the percentage of sucrose found.

The figure 1619 is the product of the expression:

$$\frac{(10 \times 66.5) + (3.5 \times 52.4)}{52.4} \times \frac{100}{1}$$

and is a constant when 10 grms. of sucrose and 3.5 grms. of lactose are present in filtrate B.

The figure 1.269 is the ratio of the specific rotation of sucrose to that of lactose, *i. e.*,  $66.5 / 52.4$

In a similar manner a formula may be calculated for use with the Milk Products Sub-Committee's method (see above).

It should be noted that the specific rotation of lactose is lowered to some slight extent during the manufacture and sterilisation of unsweetened condensed milks and the accuracy of polarimetric methods will be affected thereby. For this reason Revis and Bolton recommended the use of Fehling's method for such milks, in the 4th Edition of this work.

**Copper Reduction Methods.**—Of late years much attention has been paid to increasing the accuracy of copper reduction methods for determining lactose; these methods are, however, even in their modernised forms, strictly empirical, require the strictest adherence to detail, and are, for this reason, rather less popular than other methods. Further, these methods are open to the objection that lactose reduces more copper in the presence of sucrose than in its absence and this fact is not allowed for in the older tables. It has, however, been taken into account in the volumetric method of Eynon and Lane (*J. Soc. Chem. Ind.*, 1923, **42**, 32T; 1927, **46**, 434T), in which methylene blue is used as an internal indicator. Monier-Williams (*loc. cit.*) finds it best to carry out copper reductions side by side upon the unknown solution and a solution containing a known amount of lactose, prepared as described above, in which case he finds the amounts of cuprous oxide to be proportional to the weights of lactose present.

**Invert Sugar.**—The presence of invert sugar has been demonstrated in various condensed milks and its detection and determination have become of importance where the determination of milk solids in condensed milks is necessary. As Monier-Williams points out (*loc. cit.*), it has the effect of raising the percentage of apparent lactose, as determined by copper reduction, and of lowering that found polarimetrically, as shown by the following table:

Grm. invert sugar added to 100 grm. condensed milk	Sucrose, %	Lactose hydrate, %		Diff. between pol. and copper figures for lactose
		By pol.	By copper	
Nil	40.86	12.32	12.54	0.22
Nil	40.92	12.55	12.65	0.10
Nil	40.84	12.53	12.63	0.10
0.5	40.70	12.36	13.42	1.06
0.5	40.77	12.43	13.43	1.00
1.0	40.83	12.06	13.95	1.89

It should, therefore, be possible to detect with certainty 0.5% of invert sugar, provided that no sugars other than sucrose, lactose and invert sugar are present. The method is, however, rendered somewhat uncertain by the reduction which takes place in the

specific rotation of lactose if the milk is overheated in manufacture without a corresponding decrease in copper reducing power.

Monier-Williams states that a more certain indication of the presence of invert sugar is afforded by Barfoed's solution of copper acetate which is reduced by monose sugars, such as dextrose and lævulose, but not by dioses, such as lactose; he gives details of the method of applying the solution and calculating the proportion of invert sugar.

Hinton and Macara (*Analyst*, 1931, 56, 286) have put forward a method for the estimation of lævulose, which they have worked out in conjunction with the Society of Public Analysts' Committee. This method is divided into four stages:

1. Preparation of milk serum by one of the methods described in the Society of Public Analysts' Committee's report on the determination of sucrose.
2. Oxidation of lactose (and dextrose, if present) by treatment with alkaline iodide.
3. Reduction of copper by lævulose (in Luff's solution).
4. Determination of the reduced copper by the iodimetric method of Shaffer and Hartmann.

It has been found by applying this method that invert sugar is undoubtedly formed in sweetened condensed milks, but that the lævulose is apparently converted simultaneously to laevan which has no reducing action, only small amounts of lævulose (about 0.5%) remaining as such.

The examination of condensed milk for preservatives and the constituents of the ash may also be carried out as in the case of milk.

## SECTION D. SPECIALLY-TREATED MILKS

At one time there was a great demand for milks the composition of which had been modified either by the abstraction of certain of the constituents or alteration of their ratio by various means.

At present there are on the market so many dried milk preparations (*q.v.*) of varying composition that it has become possible to produce from them milk products of any desired composition, and consequently the call for these modified milks in a liquid condition is not so great.

Certain of these milks, however, may be referred to quite briefly, as follows:



### D. (a) HUMANISED MILK

This class aims at a reduction in the percentage of casein, combined with an increase in the percentage of milk sugar and fat, to amounts approximating to what is known as "average human milk," though it depends entirely upon the opinion of the maker what this may be.

### D. (b) DIABETIC MILK

These preparations have been put on the market in order to supply a fluid resembling milk, but practically free from milk sugar. They are usually patented preparations; one of the best-known is prepared by precipitating the casein of skim milk with acid, washing and treating with alkali. This solution of the casein is recombined with cream and certain salts, such as sodium phosphate, etc., the resulting liquid usually being prepared of such a composition that the proportions of fat, protein, etc., are about twice those of normal milk. This liquid can be sterilised without alteration and needs only to be diluted with water for use.

### D. (c) PEPTONISED MILK

This form of milk product is quite easily prepared by the action of tryptic ferments on milk. The milk is usually diluted about one-third with water and treated with the requisite quantities of sodium hydrogen carbonate and the pancreatic preparation, the milk being kept at 37° during the digestion. The treatment is naturally only very partial as, if the peptonisation be pushed too far, the milk is rendered distinctly bitter and unpalatable.

**Analysis of Peptonised Milk.**—*Qualitative test* for peptonisation: 10 c.c. of milk are mixed with 1 drop of pure acetic acid in a test-tube and heated for 5 minutes in boiling water. The curd is broken up and filtered, and the filtrate neutralised to litmus with dilute sodium hydroxide solution. The liquid is again filtered and 0.1 c.c. of 1% copper sulphate added, and 5 c.c. of 10% sodium hydroxide; a rose-red colour indicates the presence of caseoses and peptones (Biuret reaction). The writers have found that in perfectly fresh milk a slight rose-red colour is always given, even though caseoses are absent. This seems to be due to the presence of traces of lactalbumin which, in their experience, gives a reddish colour with the biuret test, and not the usually bluish-violet of native albumins. Cobalt sulphate maybe substituted for the copper sulphate and gives

a yellowish colour in the presence of caseoses and peptones, but in the opinion of the writers the original reaction is more characteristic.

**Test for the Nature of the Caseoses Present.**—20 c.c. of the milk are cleared from casein and albumin as in the above test, and the neutralised filtrate treated with an acid solution of zinc sulphate (made by saturating with zinc sulphate a mixture of 100 parts water containing 2 parts of 20% sulphuric acid) as follows:

(a) To 8 c.c. of the neutralised filtrate add 2 c.c. of the reagent. A precipitate indicates *acid albumin* which is usually absent in these cases. Filter.

(b) To 6.25 c.c. of the filtrate from (a) add 3.75 c.c. of the reagent. A precipitate = *proto-* and *hetero-caseoses*. Filter.

(c) The filtrate from (b) is saturated with zinc sulphate. Precipitate = *Deutero-caseoses*. Filter.

(d) The filtrate is treated with phosphotungstic acid (4% solution). A precipitate = *peptones and nitrogenous bases*.

**Quantitative Examination.**—The estimations of total solids, fat, ash, etc., are carried out as in the case of milk (*q.v.*).

#### D. (d) HOMOGENISED MILK

Machines are now in use which are capable of so emulsifying the fat of milk *in situ* that it no longer rises as cream, but remains evenly distributed throughout the milk. The machines are of several types, the best known being the Gaulin, Progress, Viscoliser and Hamlett machines. The milk is forced under high pressure (about 2,500 to 4,000 lb. per square inch) by pumps either between closely approximated surfaces or through fine apertures placed opposite one another so that the streams of milk meet under high pressure. The result is much the same in either case.

The method is being extensively used in the preparation of sterilised milks, condensed milks, artificial cream and ice creams. It has a limited use in ordinary milk supply, and would be more extensively used if the preliminary heating of the milk were not necessary.

By the use of the homogeniser, margarine fats may be introduced into skimmed milk and the resulting fluid has all the appearance and behaviour of ordinary milk. This is extensively employed for calf-feeding, and the writers have also examined such milk intended for human consumption.

It must be carefully remembered that the homogenisation of milk renders the centrifugal methods of estimating fat erroneous, unless the rotation be repeated at least twice, heating the tubes between each rotation. The following comparative figures are given by Richmond (*Analyst*, 1906, 31, 218).

Gottlieb	Werner Schmidt	Adams	Gerber
3.79	3.81	3.58	3.78
3.70	3.74	3.52	3.70
3.66	3.67	3.53	3.70
3.46	3.45	3.32	3.47
3.86	3.88	3.66	3.89
3.93	3.98	3.81	3.95

The Gottlieb and Werner Schmidt were not completely extracted, but an aliquot portion of the ether was taken.

Homogenised milk may be recognised usually by its microscopical appearance, the fat globules being almost uniformly of very small size (about 0.5 to 1.0  $\mu$  diameter) though some larger globules are always present, and account for the very fine layer of cream that rises even on the best homogenised milk on long standing.

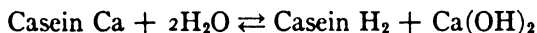
It is not easy to say that sterilised creams and dried milks have been homogenised, as the heating process largely undoes the work of the homogeniser and produces aggregations of fat globules.

According to Buglia (*Z. Chem. Ind. Kolloide*, 1908, 2, 353) the electrical conductivity of homogenised milk is greater than that of normal milk, and its viscosity is also greater.

#### D. (e) STERILISED MILK

This product in itself calls for little comment. The colour of the milk is usually somewhat brownish if the sterilisation has been at all effective, though this is improved by a previous homogenisation of the milk. A sterilised milk should be practically sterile, or the remaining organisms should have been so attenuated by the heating as to be almost incapable of development. Many brands of sterilised milk show profound change after as short a sojourn as 72 hours in an incubator at 37°. Such milks must be looked upon with suspicion, as the organisms which develop in such cases are very often capable of producing toxic substances in the milk, and their growth is often only manifested by a slight bitterness.

It may be well to point out that "boiled" milk usually means milk raised to such a temperature that it froths up, and as a rule the temperature will then be much less than 100°. For this reason it preserves its white colour, in contradistinction to sterilised milk (which has been maintained at 100° for a more or less prolonged period) in which a certain amount of caramelisation of the lactose has occurred. In fact, it may be taken as a general rule that "sterilised" milk which is white is not sterile and, for the reasons given above, may prove dangerous. Sterilised milk has no longer life than ordinary milk when once the bottles have been opened. The acidity of milk falls slightly when heated, though at the same time the concentration of hydrions increases somewhat. The pH lies between 5.30 and 5.46, though this value returns again to close on the original figure if the milk be kept. The lowering of the acidity is not caused by action on the glass of the containing vessel. A plausible explanation is that the following reaction takes place:



but Van Dam was unable to get experimental evidence in favour of this.

Analyses of the white sediment which always appears when sterilised milk is kept standing for long periods have been made by Siegfeld. The bottoms of the bottles were removed and the substance dried and washed well with ether. From 1000 c.c. 0.120 grm. were obtained and after ashing, this was reduced to 0.0235. This contained 42.4% CaO and 51.7% P<sub>2</sub>O<sub>5</sub> (the last figure is probably slightly high). Another quantity gave CaO 51.7% and P<sub>2</sub>O<sub>5</sub> 37.2%. He concludes that it is Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>.

#### D. (f) BUDDEISED MILK

A process has been devised by Budde by which milk may be sterilised by the action of hydrogen peroxide. In its latest developments the milk is treated with the peroxide for 3 hours *in vacuo* at 50 to 55° and the excess of peroxide removed by the addition of a small quantity of catalase. By this process, milk may be freed from all pathogenic and non-sporing organisms, but spores are not destroyed and the milk has therefore only the life of ordinary pasteurised milk. When properly prepared it has the taste of normal milk and of course has undergone no destructive heating.

### **D. (g) IRRADIATED MILK**

The first experiments in the application of ultra-violet rays to milk were made with the object of improving the keeping qualities, and to this end milk was exposed in thin films to the action of ultra-violet rays from quartz mercury vapour lamps. Experiments made by the writers, in an experimental apparatus with two lamps, achieved a reduction of only 88% of the organisms present, after two treatments. Subsequently, it was discovered that ultra-violet rays induced the formation of vitamin D, and illustrative of this is a patent by Steenbock (No. 236, 197) dealing with the irradiation of all substances except those entirely free from fatty matter.

Prolonged application of the treatment to milk direct has been suggested as liable to vitiate the flavour and reduce, if not entirely destroy, vitamin A. The difficulty has been overcome by a reduction of the time of treatment; or, by the alternative of the separate irradiation of ergosterol and its subsequent addition to the milk; in this way there can be no question of spoiling the flavour, and vitamin A is not endangered.

Toward the end of the year 1911, a method of reducing the lactic acid in milk was introduced by passing an electric current between special electrodes. Apart from the undesirability of obliterating lactic acid, it seems that by the process the acidity of milk can be reduced below that of freshly drawn milk, from which fact it seems certain that chemical change in the milk must be brought about.

### **SECTION E. SOUR AND FERMENTED MILKS**

These important milk products, though in their inception of Eastern origin, from time to time create a vogue in other countries. While scarcely coming within the province of the analyst, it is certain that once and again he may be called upon to give an opinion concerning them, and for this reason a general description of their nomenclature, composition, modes of preparation and bacteriology is given. This is rendered very much more easy to-day, on account of the careful and exact investigations which have been made in this subject, investigations which have successfully cleared up much that was hitherto but little understood.

Both fermented and sour milks are undoubtedly the result of natural causes in their own native home. In tropical climates it is

quite impossible to keep milk fresh and sweet, and the combination of a high temperature and dirty handling would inevitably lead to a rapid and probably dangerous decomposition, had not nature provided a safeguard in the form of lactic acid organisms of considerable activity, by the growth of which all danger is avoided. The inclusion of yeast was probably quite as accidental in the beginning, but on account of the alcoholic content so produced in the milk commending itself to the native consumer, the activity of these yeasts has been sedulously cultivated, resulting in the well-known ferments used to-day for the production of fermented milks. The steady handing on of these "starters" for generations has undoubtedly produced, by selection and environment, a number of organisms which now form a class by themselves, though exhibiting considerable pleomorphism and biological eccentricities which have of recent years been recognised.

Fermented milks are represented chiefly by Koumiss, Kephyr and Mazun, while soured milks are known as Yoghout (Bulgaria), Leben (Egypt), Gioddu (Sardinia), and Dadhi (India).

The chief distinction between the two classes is the presence of an active alcoholic fermentation of the milk sugar by yeasts present in the inoculating material, while the distinguishing characteristic of all classes of soured milks, fermented or otherwise, is the presence of a long rod-shaped organism, known under many names such as *B. caucasicum*, *B. mazun*, and more recently as *B. bulgaricus*.

This organism is distinguished by its exceptional power of converting lactose into lactic acid, its high optimum temperature of growth, and its curious "root"-like colonies on suitable media. In properties it is closely allied to *B. acidophilus* and some other organisms which seem to be part of the natural flora of the intestines.

It is to this type of organism that Metchnikoff has drawn so much attention, on account of its powerful acid production and its undoubted ability to flourish in the intestine. Whether it be the organism itself or whether it be the lactic acid produced by it, and introduced usually at the same time with the organism, which is the effective agent, must be yet left an undecided question. The acid produced by it is stated by various observers to be inactive, dextro- and lævo-lactic acid; but there is little doubt that the predominant acid is *d*-lactic acid. Statements with regard to the nature of the acid must be accepted always with reserve, on account of the

extreme likelihood of conversion during isolation. For a discussion of this point see White and Avery (*Cent. f. Bakt. Abt. ii*, 1909, 25, 161). The bacteriology of this organism is fully dealt with by the following authors:

Kuntze (*Cent. f. Bakt., Abt. ii*, 1908, 21, 737).

Luerssen and Kühn (*ibid.*, 20, 234), also

Makrinoff (*Cent. f. Bakt., Abt. ii*, 1910, 26, 374).

Together with this organism, there is present in all native soured and fermented milk the ordinary lactic acid organism known in Europe generally as *Bacillus* or *Streptococcus Güntheri*, but more properly called, as by Heinemann, *Streptococcus lacticus*. This organism, to which the ordinary coagulation of milk is usually due, has also, on account of its great pleomorphism, received a host of various names and shows a similar variation to *B. bulgaricus* in its temperature optima. It is probably of faecal origin and is also closely allied to, if not identical with, *Streptococcus pyogenes*. It is very probable that it is an accidental contamination, but finding in milk its most suitable environment it is always present as a predominant organism and may be looked on as quite characteristic of these milk products.

The distinguishing organisms of the fermented milks then are, a yeast, *B. bulgaricus* and *Streptococcus lacticus*, the last two being also the necessary feature of soured milks. From the methods of production it follows that there are always present with these a very great variety of other organisms, such as *B. acidi lactici* Hueppe, *Oidium lactis*, *Sarcina lutea*, etc., but these can only be looked upon in the light of accidental impurities and are of no value to the product.

The following brief accounts of the preparation of these products may be of interest, but for full descriptions, reference should be made to the interesting details given in a book entitled "*The Bacillus of Long Life*" by Loudon M. Douglas.

### E. (a) KOUMISS

This is a fermented milk characteristic of the Steppes of Russia and Central Asia and is made from mare's milk. The milk is warmed, usually by the addition of warm water and some old koumiss is added as a starter, the heat being retained by carefully covering up the vessel. After standing 24 hours it is violently agitated, a process repeated once again after 24 hours.

In case old koumiss is not available as a starter, this is made from ground millet which is boiled with water to a thick paste. This is added to some boiled milk and kept at about 37–38° for 24 hours till active fermentation is in progress. More milk is then added, with constant stirring for 12 hours. The resulting fluid is a weak koumiss which may be used for making a fresh batch.

The use of such a starter will undoubtedly lead to butyric acid formation if care be not exercised, and for this reason mare's milk is preferable to cow's milk on account of its greater lactose content and smaller fat percentage. Undoubtedly casein degradation does take place and it is only the thorough aeration brought about by the beating that keeps the butyric acid change in check, while at the same time lactic acid development is favoured, and the acid then protects the milk from undesirable changes.

The bacteriology of koumiss has been very carefully studied by Rubinsky (*Cent. f. Bakt.*, Abt. ii, 28, 163). The necessary organisms are: (1) The koumiss yeast and (2) the koumiss bacterium. The yeast is of an exceptional nature. It ferments lactose readily forming lactic acid to the extent of 0.36% and the milk is coagulated and at the same time it creates a peptonising action on the casein and albumin. Free fatty acids also appear as part of the activity of this organism and consequently esters arise which give the peculiar odour so characteristic of koumiss.

The product is usually styled "Weak" koumiss for 12 hours after the second beating up, and after a further 12 hours' standing it is termed "medium" koumiss and finally "full" koumiss. These distinctions simply mark certain stages in the progress of the alcoholic fermentation and acid production.

### E. (b) KEPHYR

This fermented milk is characteristic of the Caucasus districts. It is notably distinguished from koumiss in that it may be made well from cow's milk, and that the temperature of preparation is markedly lower, viz.: about 11–15.5°, whereas for koumiss a higher temperature is employed. The fermentation is started by adding to the milk the so-called "Kephyr grains" which have been well soaked in water and then several times in milk before use (each soaking taking about 2–3 hours). The milk to be fermented is boiled just before use and cooled, and the grains added, a leathern bottle usually being



employed as a receptacle, and this is well shaken every 2 hours, the milk being ready in 24–28 hours. The casein should then be in a fine flocculent condition and not lumpy and show no tendency to conglomerate. A gradual degradation of the casein takes place on keeping. The “Kephyr grains” themselves, when dry, are hard brownish granular masses. These are made up of a yeast, generally confined to the rind, and a mass of bacteria in the centre. With regard to the nature of the bacteria present and their necessity in the preparation of kephyr there is much dispute. Nikolaiewa claims that only the yeast and an organism closely allied to *B. caucasicum* are essential. *Streptococcus lacticus* is always present, but is looked on as accidental. It may be remarked at this point that the streptococcus can survive the drying in the grains for a very long period. The most careful work, however, is that of Küntze (*Cent. f. Bakt.*, Abt., ii, 1909, 24, 101). He finds present, (1) *Streptococcus lacticus*; (2) *B. acidi lactici* Hueppe and allied organisms; (3) yeasts; (4) butyric acid bacteria. Of these, the last seems to be essential. The yeast may be any ordinary variety, as the lactose is attacked in presence of the lactic acid bacteria. The fermentation is a distinctly symbiotic one: the butyric acid bacteria start well at first, but are held in check by the development of the yeasts and the lactic acid bacteria, which finally are themselves checked by the yeasts leaving these predominant for a short time, though in the end the butyric acid organisms reassert themselves. Küntze describes a particular butyric acid organism which he terms *B. esterificans* or *B. kephyr*, and states that typical “grains” are formed by this organism. It is to this organism that the protein degradation must be attributed.

### E. (c) MAZUN

This fermented milk is characteristic of Armenia. In many ways it resembles the last, but has its own characteristic aroma. It is made from milk which has been boiled and cooled and inoculated with some old mazun rubbed up with milk. A shallow dish is employed to hold the milk, which is thoroughly stirred and closely covered with a thick cloth. Well-made mazun shows no separation of whey. It is often used as a “starter” for making butter, and the curd obtained from the butter milk after pressing is mixed with flour and dried in the sun and then often used as a “starter” for fresh

mazun. Three different organisms seem to be necessary: (1) yeasts, (2) long rod-shaped lactic acid bacteria and (3) *Streptococcus lacticus*.

The yeasts like those of koumiss give rise to esters which give the characteristic odour, but protein degradation is slight as the lactic acid production is so powerful as to render such a change almost impossible.

The long rod-shaped lactic acid bacteria seem to belong to the *B. bulgaricus* group and have an optimum temperature of 37°.

For the general bacteriology see Dügge (Cent. f. Bakt., Abt. ii, 1905, 15, 577).

### E. (d) YOGHOUT

This product may be taken as characteristic of the non-fermented soured milks. It must be borne in mind, however, that yeasts are always present in these milks but the alcoholic fermentation is never pronounced. The characteristic organism is the *B. bulgaricus*, though *Strept. lacticus* is always present and in pure cultivation products seems to produce a more finely flavoured result. As made in their native countries, the milks are always practically a solid curd, which, however, is easily broken up on shaking. The milk used is boiled down to half its volume and being placed in wooden bowls is inoculated usually by rubbing the bowl with some old "soured milk." The bowl is then wrapped in cloths to maintain the necessary temperature (37-42°) till the milk has set to a firm curd. The starter used in Bulgaria is termed "Maya." The bacterial flora of these products is very mixed, but there is little doubt that the necessary organisms are the *B. bulgaricus* and *Streptococcus lacticus*.

The work of Metchnikoff has resulted in a very large number of more or less pure cultures of these organisms being placed on the market either as liquid milk cultures or in tablets of compressed dried milk or milk sugar for the home preparation of "soured" milk. It can scarcely be said that the result is altogether satisfactory except in skilled hands. The tablets, unless freshly prepared, are often quite useless, as the life of the *B. bulgaricus* in them is short and only the *Streptococcus* survives. The liquid cultures are much more reliable, but in them also the life of the *B. bulgaricus* is usually not more than 4 weeks.

Cultures are now obtainable under various trade names, such as "*Sauerin*," and if these be used within the time recommended by the manufacturer, good results may be obtained in the preparation of



FIG. 4.—*Streptococcus lacticus*.

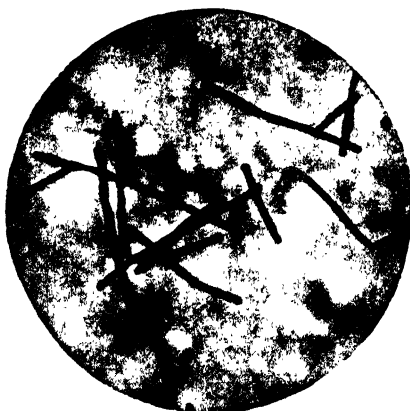


FIG. 5.—*B. bulgaricus*.

sour milk. In using any such cultures care must be taken to secure sterilisation of the milk, and for this purpose it must be boiled for at least three-quarters of an hour before using, otherwise spore-bearing organisms present in the milk will develop rapidly, outgrowing the organisms introduced by the culture, with results which may be very prejudicial to the consumer. Some of the cultures

now sold contain not only *B. bulgaricus* but also *B. acidophilus*—the latter organism having been proved to be generally associated and, as already mentioned, a natural constituent of the bacterial flora of the intestine.

The great difference between the milks so produced and the native article is that in the former the milk is not concentrated as a rule and, for convenience of transport, the curd is often beaten into a cream.

The *B. bulgaricus* seems to attack the milk sugar only, and the



FIG. 6.—*B. bulgaricus*.

statement that butyric acid is formed by it from the butter fat is, in the opinion of the writers, erroneous.

Further, it is necessary to realise clearly that such products as koumiss and soured milks prepared on the home market seldom resemble very closely the native-made article. In soured milks practically the only change is that of lactose to lactic acid and protein degradation is practically nil. Koumiss is also often made by the addition of glucose to separated cow's milk in order that alcoholic fermentation may be brought about by ordinary distiller's yeast. Such an article probably fulfils all necessary requirements and cannot be looked on as an adulterated product.

The foregoing photographs<sup>1</sup> give some typical forms of *B. bulgaricus* and *Streptococcus lacticus*.

The following analyses are by Vieth (*Analyst*, 1885 10, 24; 1886, 11, 69; 1887, 12, 43):

<sup>1</sup> The writers are indebted to the kindness of Dr. Ralph Vincent, Director of the Bacteriological Laboratory of the Infants Hospital, Vincent Sq., London, for these photographs which have been specially made from cultures in the writers' possession.

%	Full koumiss			Medium koumiss			Whey koumiss			Diabetic koumiss			Russian koumiss			Cows' milk koumiss			
	Days old			Days old			Days old			Days old			Days old			Days old			
	1	8	22	1	8	22	1	8	22	1	8	22	1	8	22	1	8	22	
Water	88.90	90.35	90.57	87.55	88.39	88.62	89.74	90.63	91.07	92.24	92.38	92.55	91.87	92.26	92.52	88.50	90.15	90.31	90.36
Alcohol	0.15	0.04	1.04	0.29	0.97	1.05	0.30	1.03	1.38	0.28	0.35	0.57	0.22	0.45	0.57	0.17	0.92	1.03	1.12
Fat	1.35	1.36	1.38	1.54	1.56	1.58	0.11	0.13	0.15	0.51	0.52	0.51	0.34	0.33	0.33	1.65	1.48	1.58	1.57
Casein	2.01	1.96	1.88	1.40	1.40	1.30	0.15	0.14	0.11	2.10	2.13	2.05	2.32	2.17	2.03	2.06	2.00	1.93	1.70
Albumin	0.30	0.23	0.20	0.43	0.25	0.14	0.39	0.36	0.32	0.30	0.25	0.18	0.08	0.07	0.07	0.32	0.22	0.21	0.09
Lactoprotein and peptones	0.34	0.53	0.77	0.48	0.76	0.97	0.44	0.49	0.58	0.36	0.48	0.65	0.32	0.48	0.63	0.32	0.56	0.74	0.91
Lactic acid	0.34	0.96	1.40	0.68	1.20	1.67	0.60	0.91	1.26	0.75	0.86	1.22	0.06	0.31	0.56	0.26	0.97	1.39	1.94
Sugar	6.03	3.10	2.18	6.80	4.70	3.90	7.48	5.52	4.34	2.78	2.42	1.64	3.95	3.08	2.45	6.16	3.14	2.23	1.73
Ash																			
Insoluble	0.41	0.34	0.35	0.49	0.45	0.44	0.42	0.42	0.42	0.37	0.37	0.37	0.38	0.36	0.35	0.42	0.34	0.35	0.33
Soluble	0.17	0.23	0.23	0.28	0.32	0.33	0.37	0.37	0.37	0.22	0.24	0.26	0.46	0.49	0.49	0.16	0.22	0.23	0.25

The following analyses of Koumiss have been published by Orloff (Russian Koumiss):

Age of koumiss	Alcohol, %	Sugar, %	Lactic acid, %	Fat, %	Protein not peptone, %	Peptone, %
12 hours.....	1.94	2.11	0.84	.....	2.24	0.28
30 hours.....	2.18	1.87	0.94	.....	2.20	0.30
40 hours.....	2.32	1.53	1.09	2.25	2.15	0.35

and the following by Allik (Caucasian Koumiss):

Age of koumiss	Alcohol, %	Lactic acid, %	Casein, %	Albumin, %	Acid albumin, %	Hemi-albumose, %	Sugar, %
62 hours .....	0.57	0.84	1.01	0.41	0.18	0.66	1.43
84 hours .....	0.67	0.91	0.95	0.40	0.19	0.56	0.98

And the following are analyses of Kephyr given by Spivak and show the alterations which take place during maturing:

	First day	Second day	Third day
	%	%	%
Fat.....	.....	1.75	1.70
Casein.....	3.34	2.87	2.99
Lactalbumin.....	0.11	0.03	0.00
Acid albumin.....	0.09	0.10	0.25
Hemi-albumose.....	0.09	0.28	0.40
Peptone.....	0.03	0.04	0.08
Lactose.....	3.75	3.42	3.09
Lactic acid.....	0.54	0.56	0.65
Alcohol.....	.....	0.80	1.00

### Analysis of Fermented Milks

The fat, total nitrogen, sugar and ash are estimated in the usual manner, except that in the case of the fat, if determinations are made by the Gottlieb process (which is the only satisfactory method for these substances), preliminary neutralisation of the acidity must be effected in the Gottlieb tube.

**Lactic Acid.**—There is no exact method of determining lactic acid; but the amount present is usually arrived at by titrating a known weight of the sample with  $N/2$  sodium hydroxide, using litmus paper

as an indicator. (1 c.c. of  $N/2$  NaOH = 0.045 grm. lactic acid.)

**Alcohol.**—About 100 grm. of the fermented milk are diluted with an equal volume of water and 100 c.c. distilled off. The distillate is neutralised with  $N/10$  barium hydroxide and redistilled till 50 c.c. have passed over, and the alcohol estimated from the gravity of this last distillate.

**Volatile Acidity.**—A further quantity of water is added to the residue from the *first* alcohol distillation and the distillation continued till 50 c.c. of the distillate require only about 0.1 c.c.  $N/10$  sodium hydroxide for neutralisation. The total distillate is then titrated with  $N/10$  barium hydroxide and to the amount so found are added the number of cubic centimetres required to neutralise the first alcoholic distillate. The volatile acidity is usually expressed as acetic acid.

**Casein.**—This can be directly filtered off and washed, and the nitrogen estimated.

**Lactalbumin.**—This cannot be found very accurately, but is usually estimated by neutralising the filtrate from the casein, acidifying with 0.1% of acetic acid and boiling the solution. The precipitate is taken to be lactalbumin, the nitrogen being estimated in it after filtration and washing.

**Albumoses and peptones** are found by deducting the sum of the nitrogen in the above constituents from the total nitrogen, or the filtrate from the albumin may be neutralised, cautiously evaporated to a convenient bulk, saturated with zinc sulphate, and the precipitate filtered off, washed with saturated zinc sulphate solution and the albumose nitrogen estimated in the precipitate. The peptone nitrogen is obtained by difference.

## SECTION F. INFANTS' FOODS

The great multiplicity of artificial preparations classed under Infants' Foods can hardly be included under the heading of milk products, as a very large number is produced from cereals and similar substances, and only occasionally contains actual milk derivatives.

These preparations may be divided into three main groups:

(1) Dried milk powder with which has been incorporated starch, malt extract and sugars other than lactose;

- (2) Humanised milk powder;
- (3) Milk preparations for special cases of malnutrition.

(1) These foods are mixtures of whole milk or half cream milk to which starch or other similar material has been added in such a manner as to cause it to become hydrolysed when prepared for consumption and act as a protective colloid to the casein against the action of acid; the casein is thus precipitated in a fine flocculent form and not as a heavy curdy mass.

(2) During recent years the manufacture of this class of preparation has become an important industry, the general underlying principle of which has been to produce foods which will replace human milk as the natural food of the infant. Analytically the composition of these preparations, in terms of fat, protein, carbohydrate and ash, is so similar to human milk, as to give an impression of their being identical substances. All the necessary ingredients of these substitute infant foods are available in cow's milk, and the manufacturers have different methods of modifying cow's milk in order to effect the proper balance of the various constituents.

(3) The chief product of this class is whey in powder form. Apart from its value as a constituent in the modification of cow's milk for infant feeding, it is a valuable and reliable nutrient for gastric and intestinal disorders.

The methods of analysis usually employed are simply those which estimate the various constituents in a similar manner to the analysis of feeding-stuffs and give no idea of the method of manufacture, or of the condition, or digestibility of the components. A systematic investigation of a large number of infants' foods by methods which give to some extent the desired information has been carried out by Julian L. Baker and H. F. E. Hulton. As the various estimations themselves are not pertinent to this section, only the lines of investigation are given without entering into the full analytical details. In the preparation of the sample and in the interpretation of the results of analysis care must always be taken to bear in mind the directions prescribed for making up the food, as these may produce profound changes in its nature.

The following are some of the estimations suggested by Baker and Hulton:

**Starch.**—This is estimated by a direct polarimetric method, as originally devised by Lintner, and modified by Thorne and Jeffers



(*Analyst*, 1909, 34, 322). The readings must be corrected for other optically active substances found. In cases where much starch is present information whether it has been heated or not may be obtained by estimating the starch as above, and also in that part of the food which is insoluble in warm water. In the *absence* of diastase, the difference gives information as to the amount of starch rendered soluble by heating. When diastase is present the material must be extracted with ammonia (80 c.c. of *N*/10 diluted to 1,000 c.c.) in order to stop its action.

**Hydrolysed Starch Products.**—These, when present, cannot be directly estimated, but from the sp. gr., copper reduction and rotation of the aqueous extract, a very fair idea can usually be obtained of the proportions of these substances after making allowance for other soluble substances, such as cane sugar, lactose and soluble proteins, when present. The aqueous extract of the food is made by shaking a suitable quantity—from which fat has been extracted if necessary—with distilled water for 2 or 3 hours.

**Reducing Sugars. Dextrose.**—In the absence of starch conversion products and lactose, the copper reduction of the cold water extract may be taken as due to dextrose or invert sugar. In the presence of maltose the estimation of dextrose becomes difficult, but there is little probability of dextrose being present in the ordinary cereal foods, except in very small quantities. In presence of lactose, the copper reduction should be corrected by separately estimating this sugar. Baker and Hulton have shown (*Analyst*, 1910, 35, 512) that for the estimation of lactose very fair results can be obtained by fermenting away the other sugars by means of yeast, and estimating the lactose in the residual liquid. For this purpose the aqueous extract of the material is boiled with 2% of citric acid to invert any cane sugar, neutralised, and a small quantity of cold water extract of diastatic malt added. About 0.5 gm. of washed brewers' yeast is then added, and the whole incubated for 3 days at 27°. The liquid is then cleared with alumina cream, filtered, boiled, and titrated against Fehling's solution. When hydrolysed starch products are present the estimation of the lactose will give slightly too high a value, on account of the presence of unfermentable reducing substances derived from malto-dextrins, but, as a rule, the error may be neglected.

**Cane Sugar.**—This may be estimated in the cold water extract by heating with 2% of citric acid in boiling water and the increase in the copper reduction estimated. But where any considerable amount of cane sugar is present it should be inverted by digestion with a little washed yeast for 4 hours at 55°, the solution cleared and polarised and the cane sugar calculated by the usual Clerget formula.

**Protein.**—The nitrogen is estimated in the usual manner by the Kjeldahl-Gunning method. The factor 6.25 is suitable, though necessarily only approximate, on account of the diversity of proteins which may be present. The identification of the various proteins is, owing to the nature of the case, very complex.

**Fat.**—This is estimated by the Röse-Gottlieb method, with all the precautions given under dried milk, or by the Bondyzynski method, as described under cheese (see page 204). Ether extraction is unsuitable in the case of foods containing dried milk.

**"Cellulose."**—Baker and Hulton include under this heading those substances of a carbohydrate nature, which are insoluble in boiling water and are not attacked by diastase. In order to estimate the actual indigestible substances a pancreatic digestion would be necessary, but, to avoid this, these investigators have devised the following method: 5 grm. of the material—from which the fat has been extracted if necessary—are made into a paste with cold water, 200 c.c. of water added, the whole heated to boiling, and the boiling maintained for 30 minutes. The solution is then cooled to 80° and 5 c.c. of malt extract (prepared by extracting a diastatic malt, with 3 times its weight of cold water for 1 hour and filtering clear) added, and after 5 minutes again boiled, cooled to 60°, 25 c.c. further of the malt extract added, and the whole digested at this temperature for 3 or 4 hours. The solution is then filtered through a tared filter paper, and the residue washed repeatedly with water at 60° till free from sugar, then with alcohol and ether and finally dried to constant weight. The nitrogen in it is then estimated on the residue, and the protein present calculated and deducted. In a parallel experiment the ash in the weighed residue is estimated, and its amount is also deducted. The final residue is returned as "cellulose."

**Moisture and Ash.**—These are estimated in the ordinary way by drying and ignition.

**Saccharifying Diastase.**—This is determined by Lintner's method as modified by the Institute of Brewing (*J. Inst. Brewing*, 1906, 12, 6).

**Liquefying Diastase.**—This is estimated by observing the change in viscosity produced in 200 c.c. of a 2% potato starch paste in 10 minutes at 20° under the action of 10 c.c. of a 5% cold water extract of the sample.

**Alkalinity.**—A definite volume of the cold water extract is titrated with *N*/50 sulphuric acid, using methyl orange. An alkalinity of more than 0.15%, calculated as acid sodium carbonate, would appear to point to the addition of alkalies.

**Nature of Starch Present.**—This is determined by microscopical examination.

A consideration of the results so obtained will nearly always indicate the nature of the constituents of the food and whether the claims of the makers can be substantiated.

In those foods which consist entirely of milk constituents the methods given under dried milks will apply, and in many cases in which lactose is present, and there are no added proteins, the relation of fat, protein and lactose to one another will often be a guide to the presence of dried milk. In such cases the presence of casein may be easily demonstrated.

Food	Water, %	Protein, %	Fat, %	Carbohydrate, %	Mineral substance, %	Cold water extract, %	Crude fibre, %	Remarks
Glaxo .....	3.2	24.1	26.6	40.5	5.6			Desiccated milk with added lactose.
Lactogen .....	2.7	25.0	26.2	40.4	5.7			Desiccated milk with added lactose.
Horlick's Malted Milk .....	5.1	12.5	7.9	70.9	3.6	74.5	Nil	Desiccated milk and desiccated malt extract.
Bacchus Marsh Malted Milk .....	3.7	11.2	10.8	71.2	3.1			Desiccated milk and desiccated malt extract.
Mellin's Food .....	5.6	10.2	0.9	80.6	2.7	82.8		Desiccated malt extract.
Benger's Food .....	5.4	10.9	0.8	81.9	1.0	16.8	Nil	Wheat flour and pancreatic extract; very little unchanged starch when made as directed.
Savory and Moore's .....	6.7	9.8	1.2	81.5	0.8			Wheat flour and malt extract; most of starch changed when prepared as directed.
Allenbury's Malted Milk .....	4.9	9.7	0.8	83.5	1.1			Wheat flour and malt extract; most of starch changed when prepared as directed.
Nestle's Milk Food .....	3.3	12.8	4.1	78.1	1.5	66.0	0.2	Desiccated milk, baked wheat flour, and cane sugar.
Neave's Food .....	3.2	13.4	1.2	81.1	0.7	6.6	0.4	Baked wheat flour.
Australian Groats .....	3.3	9.8	7.2	78.4	0.8		0.5	Ground oats.
Robinson's Groats .....	7.6	12.3	6.8	70.4	1.5		1.4	Ground oats.

The analyses on p. 168 of infants' foods in Queensland were made by J. B. Henderson and appear in the report of the Government Analyst for Queensland for the year ending June 30th, 1924 (*Analyst*, 1925, 50, 21).

The following analyses, published by the makers, are typical of "humanised" milk foods:

	Humanized Trufood, %	Glaxo, %
Fat.....	28.97	26.0
Lactose.....	52.25	42.3
Casein.....	6.72	{ 22.9
Lactalbumen.....	5.10	
Milk salts.....	5.48	5.6
Water.....	1.48	3.2

A comparison is shown below between these preparations reconstituted according to instructions, cows' milk and human milk:

	Humanised Trufood, %	Glaxo, %	Cows' milk, %	Breast milk, %
Fat.....	3.45	3.25	3.50	3.30
Lactose.....	6.25	5.25	4.70	6.50
Casein.....	0.80	{ 2.70	3.00	0.90
Lactalbumen.....	0.60		0.30	0.40
Milk salts.....	0.65	0.70	0.80	0.20
Water.....	88.25	88.10	87.70	88.70

The following analysis shows the composition of whey in powder form, known as "Secway" manufactured by the Trufood process:

	%
Fat.....	1
Lactose.....	76
Whey proteins.....	13
Milk salts.....	9
Water.....	1

## SECTION G. DRIED MILK

The production of dried milk has now become an established and important industry, chiefly through the great improvements in the manufacturing process.

There are really only two methods in use for effecting the production of dried milk; (1) drying on steam-heated rotating drums, (2) spraying into a chamber through which a current of warm air is passing.

The former, or drum process, was first introduced by Hatmaker, and all processes involving drying on drums are really modifications of the Just-Hatmaker process.

In the original process the milk is run between two drums, separated by about  $\frac{1}{8}$  in., heated to a temperature of  $100^{\circ}$ , and the dried milk is scraped off in a thin film by cutting edges after the film has passed over about  $\frac{3}{4}$  of the surface of the drum. In the Ekenburg-Passburg method the drum rotates *in vacuo* and a temperature of  $110-120^{\circ}$  is employed. In the Nicolai method, the milk is first condensed and run in a fine film on to a rotating drum heated by steam and the film is continuously removed, raised by an elevator and allowed to fall into an apparatus which completes the removal of the water. The product is then sieved. In the Gabler process (much used in Switzerland) the milk is pasteurised and then concentrated in the vacuum pan, strongly cooled and led on to the drum, the dried milk as it is scraped off being carried by an endless band to the sieves, while a strong current of air passes over it and also over the drying drum.

The principle of the spraying process is essentially different. The milk is pumped through fine orifices into a chamber through which a rapid current of warm air passes and arrangements are made to catch the powder which is so produced. The process seems to have originated with Stauf, who proposed to spray whole milk and to employ an air current of about  $80^{\circ}$ . The object of all these spray processes is to obtain a more soluble product without the coagulation of the albumen or destruction of the enzymes. In the Bevenot process a much finer spray and consequently much higher pressure is employed, and the temperature of the air current is only a little below  $100^{\circ}$ . In the Krause process (a German process, much used in Italy) the milk is sprayed by centrifugal force, being fed on to a flat plate which revolves at high speed. The Dick process is a modification of this centrifugal spray process. A method of drying which combines the hot drum and spray processes, known as the Vacuum spray film dryer, is used in America; this is a modification of the Ekenburg-Passburg method, the milk being sprayed through the vacuum zone

onto the revolving drum. The milk thus becomes partially dried before it reaches the drum, and the film on the drum is more easily controlled at the required uniform thickness. The best-known form, however, is the Merrell-Soule process in which concentrated milk is sprayed into a chamber through which an air current at 150–200° is passing. In spite of the high temperature employed, the evaporation is so rapid that albumin is not coagulated and enzymes are not destroyed. The product is quite soluble in cold water, the taste of the resulting milk being but slightly different to raw milk, and the fat emulsion is perfect. The process is also applied to the production of separated milk powder, whey powder, etc. The yield is practically theoretical, 8 litres of milk giving about 1 kilo of dried product. The spray process has been the subject of extensive research in this country and in U. S. A., and the development of the process is reflected in many ways by the quality of the finished products.

Dried milk powders produced by the spray process have greater solubility than those made by the hot drum process, being practically 100% soluble in hot water.

Hunziker (*Condensed Milk and Milk Powder*, 4th edition), discussing this subject, gives figures to show that only 69.61% of drum-dried skimmed milk is soluble in cold water at 78.5° F., and 78.09% soluble in hot water at 210° F. The corresponding figures for spray-dried skimmed milk are given as 99.12% in cold water and 101.76% in hot water.

The addition of alkalies and salts to the milk in the earlier stages of the industry, in order to ensure the satisfactory solubility of the protein in the dried product, is now largely omitted. The processes have made such progress that these substances are now no longer required.

If a solution of milk powder is alkaline to litmus it is certain that alkali has been employed in its preparation, but this test will only apply to fresh preparations or those which have been carefully protected from contact with the air, as otherwise a considerable rise in acidity takes place.

This is illustrated in the table on p. 172.

The ash from 5 grm. of dried milk powder, as a rule, requires about 0.5 c.c. of *N*/10 sulphuric acid to render it neutral to litmus.

	Cubic centimeters of $N/1$ - NaOH per 100 gm. powder to phenolphthalein		Ash from 5 gm.	Reaction of ash to litmus
	Fresh	After 5 months		
Cream powder (1)....	10.5	30.0	0.204	Alk.
Cream powder (2)....	11.0	11.5	0.238	Alk.
Whole milk (1)....	10.5	42.0	0.289	Alk.
Whole milk (2)....	12.5	12.5	0.291	Alk.
Skim milk (1)....	8.0	11.5	0.389	Alk.
Skim milk (2)....	16.0	16.5	0.399	Alk.

The keeping properties of dried milk powders have been the subject of much discussion and research. The general belief that drum-dried milk keeps more satisfactorily than spray-dried milk has, from prolonged and intensive examination, proved somewhat fallacious, as many exceptions on both sides have been experienced. The most striking evidence of deterioration is the development of a peculiar flavour, known in the trade as "tallowiness." Palmer and Dahle (*J. Dairy Sci.*, 1922, 5) suggest that the presence of occluded air within the granules of the powder made by the spray-process has an important bearing on the fact that this type of whole milk powder is especially prone to develop "tallowiness"—i. e., oxidative deterioration. Holm and Greenbank (*ibid.* 1923, and *Proc. World's Dairy Congress* 1923), on the other hand, have shown that this suggested susceptibility to oxidation and the "tallowiness" produced are quite exceptional to the process by which the milk is produced and attribute the fault to an autoxidation process. They have shown that small quantities of oxygen will produce "tallowiness" and that autoxidation, once started in any powder, proceeds rapidly.

There are undoubtedly changes which take place in dried milk on keeping. These affect its solubility sometimes profoundly. There is also the development of rancidity from which few dried milks, other than skim milk, are at present free. These changes are sometimes attributed to bacteria, but much doubt attaches to such an explanation. With whatever care drying may be done, there is necessarily a change in the state, if not in the chemical composition of the milk, and rancidity especially would seem to be due to an oxidation started during the drying process and continued in a catalytic manner during storage of the dried product.

**Standards and Standard Definitions.**—Regulations controlling the sale of dried milks have been in force in England since 1923. These regulations (*Statutory Rules and Orders*, 1923—No. 1323) apply to “dried milk to which no other substance has been added and to the dried milk contained in any powder or solid of which not less than 70% consists of dried milk.”

Unless the dried milk is contained in a tin or receptacle whose gross weight exceeds 10 pounds, the tin must be labelled in a prescribed manner and the dried milk must contain not less than the following percentages of milk fat:

In the case of milk described as dried full cream milk not less than 26%;

In the case of milk described as dried three-quarter cream milk not less than 20%;

In the case of milk described as dried half cream milk not less than 14%; and

In the case of milk described as dried quarter-cream milk not less than 8%.

The tin must also bear a declaration of the equivalent of its contents in pints of milk.

If sugar or other substance is added to the dried milk the nature of the added material must be specified upon the label in a prescribed manner. The terms “milk,” “three-quarter cream milk,” “half cream milk” and “quarter cream milk” mean milk containing not less than the following percentages of milk fat and milk solids:

	Milk fat	Milk solids (including fat)
Milk.....	3.6	12.4
Three-quarter cream milk.....	2.7	11.6
Half cream milk.....	1.8	10.8
Quarter cream milk.....	0.9	9.9

“*Skimmed milk*” means milk which contains not less than 9% of milk solids other than milk fat.

Tables have been prepared by Hinks (*Analyst*, 1924, 49, 471) and Henville (*Analyst*, 1924, 49, 472) to assist in the calculation of the number of equivalent pints of fresh milk from the weight of the contents of the package and the analysis of a dried milk.



The United States Department of Agriculture defines dried milk as the product resulting from the removal of water from milk and containing not less than 26% of milk fat and not more than 5.0% of moisture; dried skimmed milk is the product resulting from the removal of water from skimmed milk and contains not more than 5% of moisture.

**Analysis of Dried Milks.**—The following are analyses of dried milk powders:

## WHOLE MILK

Water	Total solids, %	Fat, %	Protein, %	Ash, %	Fat Protein	Source
2.50	97.50	28.30	32.10	5.34	0.78	England.
1.40	98.60	29.20	26.92	6.00	1.08	France.
6.39	93.61	27.35	27.48	6.00	0.99	France.
3.30	96.70	23.97	26.38	6.19	0.91	France.
6.03	93.97	25.60	23.84	6.44	1.07	France.
5.51	94.49	23.75	24.71	6.49	0.96	Austria.
5.65	94.35	23.42	25.48	6.46	0.92	Holland.
5.29	94.71	26.55	25.17	5.57	1.05	Algäu.
4.23	95.77	29.50	26.57	5.80	1.11	N. America.
4.43	95.57	25.97				Germany.
6.73	93.27	30.23	22.75	5.50	1.33	Holland.

## HALF CREAM MILK

Water	Total solids, %	Fat, %	Protein, %	Ash, %	Fat Protein	Source
5.01	94.99	15.26	38.39	6.67	0.40	Belgium.
5.00	95.00	15.10	33.30	6.00	0.45	France.
4.65	95.35	17.06	29.43	6.78	0.58	.....

## SKIM MILK

Water	Total solids, %	Fat, %	Protein, %	Ash, %	Lactose Protein	Source
3.00	97.00	0.75	34.00	7.00	.....	America.
6.29	93.71	1.02	35.45	8.17	1.21	America.
13.19	86.81	1.40	28.89	7.78	1.68	Germany.
13.88	86.12	3.60	30.30	7.75	1.15	Holland.

## SWEET WHEY

Water	Total solids, %	Fat, %	Protein, %	Ash, %	Lactose Protein	Source
2.20	97.80	1.66	14.16	8.20	5.22	America.
2.10	97.90	1.60	13.72	8.80	5.38	America.

The above values are all selected from tables given by Burr (*Milch. Zent.*, 1911, 7, 118).

An extensive investigation into the methods of fat estimation in dried milk has been made by Utz (*Milch Zentralbl.*, 1914, 43, 113). His conclusions are similar to those arrived at for cheese, and he recommends the Polenske method (see cheese) as the best and quickest. The following analyses are also due to him:

Description	Water, %	Ash, %	Fat, %	Protein, N $\times$ 6.37 %	Sugar, (by diff.) %
Whole milk.....	7.28	5.44	27.72	24.33	35.23
Whole milk.....	4.14	4.94	37.50	21.40	32.03
Whole milk.....	7.19	5.65	27.41	25.54	34.21
Whole milk.....	6.43	5.38	29.71	24.33	34.15
Whole milk.....	6.33	5.23	26.50	24.97	36.97
Half cream milk.....	7.31	5.85	20.30	27.65	38.89
Half cream milk.....	8.00	6.26	14.80	28.99	41.95
Half cream milk.....	6.60	6.26	14.65	28.54	43.86
Skim milk.....	9.04	6.96	1.07	30.58	52.35
Skim milk.....	10.31	7.14	1.92	32.81	47.82
Skim milk.....	9.05	6.87	0.63	33.25	50.20
Cream.....	3.65	3.96	51.37	17.65	23.37
Cream.....	4.17	4.21	43.80	18.98	28.84

These are all probably of German origin.

The following are typical analyses of dried milk powders and illustrate the differences likely to be found between drum and spray-dried milk powders:

	Process	Fat, %	Protein, %	Lactose, %	Ash, %	Water, %
Full-cream.....	Spray	27.5	23.5	41.0	6.0	2.0
	Drum	29.2	24.8	35.7	5.6	4.7
Half-cream.....	Spray	13.86	32.1	45.1	7.09	1.85
	Drum	16.4	28.5	43.6	6.6	4.9
Skimmed.....	Spray	1.1	34.0	54.85	7.25	2.8
	Drum	2.1	31.8	54.5	8.2	3.4

**Citric Acid in Dried Milk.**—Steuart (*Analyst*, 1924, 49, 465) has shown that full-cream milk powder contains an average of 1.16% of citric acid, whilst the average figure for skim milk powder is 1.55%. In each case this is equivalent to a proportion of 0.144% in the original milk. He found in a sample of fresh milk (April) 0.158% citric acid.

**Solubility of Dried Milks.**—Lampitt and Hughes (*Analyst* 1924, 49, 176) have studied the solubility of dried milks. These authors point out that the method of Hunziker (*Condensed Milk and Milk Powder*, p. 321) and that used at the Government Laboratory (*Food Reports*, 24, p. 171) fail in certain directions, and put forward a method which is satisfactory. This method is as follows:

(1) To about 38 c.c. of distilled water at 20° in a flask of 250 c.c. capacity add 5 grm. of full cream milk powder, or to 45 c.c. of water add 5 grm. of skim milk powder; cork and shake steadily for three minutes. Transfer the whole contents to a tared centrifuge tube, and whirl at about 1500 revolutions for three minutes. If full cream powder has been taken, now remove any layer of cream on the surface of the milk in the tube, and wipe any cream off the inside of the tube.

(2) Taking care not to disturb the deposit in the tube, pipette off about 5 c.c. of the liquid into a tared nickel dish of weight ( $w$ ); weigh rapidly; say ( $a$ ) = weight of dish plus fluid; dry for four or five hours in the steam oven, or about 5 minutes on the Mojonner hot plate, and then for 15 minutes in the Mojonner vacuum oven; weigh: say ( $b$ ) = weight of dish plus milk solids.

$$(a - b) = \text{weight of water lost.}$$

$$(b - w) = \text{weight of solids.}$$

(3) Now decant as much as possible of the fluid without disturbing the residue, wipe off any cream, etc., on side of tube, and weigh the tube plus residue plus the small quantity of associated fluid: say weight of contents = ( $c$ ). Then wash out the residue by means of a wash bottle into another tared dish. To hasten drying, alcohol may be used to wash out the deposit. If the amount of insoluble matter is small, it may be dried in the tube instead of being washed out.

Dry in oven as in (2) and weigh.

Say ( $d$ ) = weight of solids.

Then  $(c - d) = \text{weight of water lost.}$

**Calculations.**—Assume butter fat =  $Y$  per cent.; moisture =  $Z$  per cent.

Dissolved solids in the fluid contained in  $(c)$

$$= (c - d) \times \frac{b - w}{a - b} = f.$$

Therefore, weight of insoluble solids contained in  $(c)$

$$= d - f = s.$$

Whence, insoluble matter per cent. of powder =  $20s$ , and solubility of the powder =  $(100 - 20s)$ , or,

Insoluble matter per cent. of solids-not-fat

$$= \frac{20s \times 100}{100 - (Y + Z)},$$

and solubility of solids-not-fat

$$= 100 - \frac{20s \times 100}{100 - (Y + Z)}$$

In a subsequent paper Lampitt, Hughes and Bushill (*Analyst*, 1931, 56, Dec.) describe an extension of the investigation which resulted in the above method and show how the solubility varies with such factors as the moisture content of the powder. The original paper should be consulted for further details.

**Analysis of Dried Milk.**—The sample should be carefully mixed.

**Moisture.**—This is estimated by drying 1 to 2 grm. in a platinum dish to constant weight in a water oven.

**Ash.**—The dry sample from the moisture estimation is burnt (preferably in a muffle) at a very low red heat.  $\text{CaO}$ , and  $\text{P}_2\text{O}_5$  may often require to be estimated in order to detect the addition of sodium phosphate and calcium succate.

**Fat.**—This is best estimated by the Gottlieb process. About 0.5 grm. are weighed into the special apparatus and 4.5 c.c. of hot water added and the whole mixed well with a rod and 0.5 c.c. of ammonia (sp. gr. 0.925) added, mixed, and 5 c.c. of strong alcohol run in and the apparatus floated in hot water until complete solution takes place, which occurs rapidly. The estimation is then finished as in the case of cream. With very old samples of dried milk in which the fat may have become appreciably hydrolysed, the Bondzynski method, as described under *Cheese*, may be used.

**Sugar.**—10 grm. are worked up in a mortar with warm water and a little ammonia added (both to assist the solution and to destroy birotation). The paste is washed into a 100 c.c. flask, cooled, and the solution treated exactly as under condensed milk, the necessary corrections being obtained from the fat and protein estimations.

As calcium saccharate sometimes appears in dried milks, cane sugar may also be estimated.

**Protein.**—This is estimated on 0.5 to 0.7 grm. by the usual Kjeldahl-Gunning method, using the factor 6.38.

Richmond has pointed out (*Analyst*, 1906, 31, 219) that analyses of dried milk seldom add up to 100%, and his explanation that it is due to a part of the sugar being present as monohydrate is undoubtedly correct to a certain extent, but the same observer further points out (*Analyst*, 1910, 35, 516) that when milk is cleared with ordinary acid mercuric nitrate, the precipitation of albumin is not complete, and in the case of dried milks has shown that the milk sugar may be under-estimated by 1% polarimetrically. If, therefore, very accurate estimations are required, it is advisable to employ the method recommended by him of adding to every 100 c.c. of the filtrate from the acid mercuric nitrate, 5 c.c. of a 4% solution of phosphotungstic acid and 5 c.c. of 50% sulphuric acid. The solution is filtered before polarisation, and allowance is made for the extra dilution.

## SECTION H. CHEESE

This most important milk product is formed from the casein of the milk usually by the action of rennet, the curd or paracasein so obtained carrying down with it nearly all the fat present in the milk. By variations in the after treatment of the curd mass arise an extraordinary multiplicity of products, all included under the title of cheese. The finished substance is of a highly complex nature, on account of the profound protein degradation that usually takes place during ripening. The nitrogenous substances range from the original paracasein down to ammoniacal salts, and together with these are milk fat, some lactic acid, mineral constituents, added salt and colouring matter.

Cheeses may be roughly classed under two headings:

(1) *Hard cheeses*, such as Cheddar, Cheshire, Dutch, Stilton, Gorgonzola, etc., and

(2) *Soft cheeses*, such as Camembert, Pont l'Evêque, Roquefort, Brie, etc.

The two classes are roughly distinguished by the fact that in hard cheeses, the whey has been separated as completely as possible; while in the soft cheeses, it has been much retained. As a result, hard cheeses ripen slowly and keep for long periods, while soft cheeses ripen rapidly and must be consumed as soon as they have matured sufficiently. Generally speaking, the ripening process is "enzymic" in the case of hard cheeses, and "bacterial" in the case of soft cheeses. On account of the superior keeping quality of the hard varieties they naturally form the bulk of the cheeses of commerce.

The large majority of cheeses are produced from cow's milk, but in certain parts of the world the milk of sheep and goats is also employed, and there is no reason why any milk should not be used. It is interesting to note that a cheese-like substance may be made from purely vegetable materials. For instance, among the inhabitants of the Soudan, a cheese is made from the seeds of the "*Parkii Africana*." The seed kernels are used and the product is free from starch. The fresh white cheese is called "*Afiti*" and the ripened product "*Dana-Dana*" and has then the consistency of a hard cheese, possessing an aromatic smell and bitter taste. Bacteria evidently take part in the ripening, and the course seems to be much the same as in soft cheeses.

Busse (*Cent. f. Bakt.*, Abt. ii, 1905, 14, 480) has examined a cheese made from the seeds of "*Treculia Africana*" and called "*Pembe*." Cayenne pepper is added at the time of manufacture. The "cheese" becomes strongly acid by the development of lactic acid organisms and so decomposition is prevented.

The large quantity of fat present in these seeds is an important factor in the cheese-like consistency and flavour obtained. A similar type of cheese is also made in Japan from Soya beans.

The fat present in cheese is usually the fat of the milk used, but cheeses are often made from skimmed milk with the addition of margarine fats and sometimes of coconut oil (in certain Swiss cheeses), the fats being emulsified with the milk before manufacture. Such cheeses are known as "filled" or "margarine" cheeses and are a legitimate product, if their nature is clearly disclosed. In cheeses made from unskimmed cow's milk the fat should always be in excess of the protein, but this rule does not hold good in the case of cheeses

made from the milk of animals other than the cow, and it must be borne in mind that each locality has its own methods of manufacture and the composition of the cheese must be judged accordingly, and not by any arbitrary standard.

The following paragraphs give a rough outline of the method of manufacture of the two main varieties of cheeses.

**Manufacture of Hard Cheese.**—Of these cheeses, Cheddar may be taken as a type.

Perfectly clean milk is first ripened by means of a lactic acid culture. The proper development of acidity is perhaps one of the most important steps in the process. When the acidity is about 0.20% the temperature of the milk is brought to 84–86° F. and the rennet added, sufficient being added to produce coagulation in half an hour; the contents of the vat being gently stirred till coagulation sets in. After this the milk is allowed to stand undisturbed until the curd has hardened sufficiently for “cutting.” The curd is then “cut” with a wire knife lengthwise and crosswise, so that the whole is divided into small cubes. The cutting allows of the escape of whey and the contraction of the curd cubes, this result being known as “firming,” and the curd mass is gently stirred in order to prevent the cubes sticking together. It is then gently heated to about 100° F., the rate of heating being regulated by the acidity of the whey found on “cutting.” When the acidity of the whey has increased to, say, 0.25%, it is run off from the curd, gentle stirring being again employed during the process. As soon as the bulk of the whey has run off the curd is “cheddared” by being piled along each side of the vat, so that free drainage is possible, and as the whole gradually sinks into a more or less solid mass, it is cut into blocks and re-piled. When this process is complete the curd is “milled” or broken up into small uniform pieces preparatory to “salting.” After the salt is completely dissolved by the curd it is cooled and “pressed” in cotton cloths placed in the “hoops,” the object of pressing being to weld the curd into a compact and solid mass, and to prepare the cheese for the final “ripening” process.

Since Sammis and Bruhn (*Wisc. Expt. Station Res., Bull.* 27, 1912) and Benson and Cram (*J. Board Agric. Gl. Britain*, 20, No. 4, 1912) investigated the possibilities of pasteurising milk for Cheddar cheese-making a great deal of interesting work has been done by other investigators. Quite recently Price and Peckett (*J. Dairy Sci.*, 11)

have shown that milk, which has been pasteurised by any method, produces a higher scoring cheese than the identical milk not pasteurised. Three methods of pasteurising were tried, "Flash" 160–165° F., "Flash-Holder" 145–150° F., "Holder" 145° for 20 minutes. The "Flash" method was not so effective as the others. It was found that no calcium chloride or hydrochloric acid was necessary to stimulate rennet coagulation. The yield of cheese was increased from 2.5 to 4% in excess of the yield obtained from the identical milk not pasteurised.

**Manufacture of Soft Cheese.**—The procedure is essentially different from that described above, and on account of the great diversity of this type of cheese, no two processes are similar. The differences lie principally in the fact that the rennet is added to perfectly fresh sweet milk, the coagulum is carefully ladled into the shapes or hoops, and drainage is natural and without pressure, and without heat. The resulting cheese is consequently soft, and on account of the quantity of whey left in, ripening is very rapid and far more complex than in the case of hard cheese, and the flavour depends almost entirely on the particular moulds and bacteria employed.

**Manufacture of Cream Cheese.**—Cream cheeses are usually styled "Double cream" or "Single cream." The former is made from a thick cream of 45–50% of fat, and without the use of rennet. The cream is cooled to 60° F. and, if desired, a little lactic acid starter added. After standing for 12 hours the cream is allowed to drain in dry cloths under slight pressure. Single cream cheese is made from cream of 25% fat content, and a little rennet is added with the starter. If sweet cream cheese is desired, the starter is omitted.

Much discussion has taken place in recent years with regard to the standard that should be expected in cream cheese, chiefly following upon a practice that has arisen of selling soft cheeses as cream cheese. In the course of a discussion upon the subject (reported in *The Analyst*, (1924, 49, 270): Dr. J. A. Voelcker made it clear that, from the point of view of the agriculturalist, "cream cheese" meant a cheese made from cream and nothing else, while other soft cheeses were not entitled to this description.

Hodgson (*Analyst*, 1924, 49, 270) published analyses of 26 samples of cream cheese, the fat content of which varied from 1.06% to 66.03%. He suggested that any standard which may be set up for



the fat in cream cheese should also bear a reference to the protein content.

**Manufacture of "Process" or Crustless Cheese.**—This type of cheese has become very popular since the war. One of the best known brands in this country is the Canadian "Kraft" cheese. The process is merely one of mixing a ripe or semi-ripe cheese, according to the type required, with an additional quantity of water. A small quantity of emulsifying or "cooking" salt, generally disodium phosphate, is added and, if a coloured cheese is required, some colouring fluid, probably annatto. The cheese is first minced in a machine very similar to a sausage machine, and then passed to the cooker, generally a semi-spherical steam-jacketted pan which is also fitted with a mechanical agitator. Here the ingredients, mentioned above, are mixed intimately together at a temperature of about 100° F. During the early part of the heating the fat separates, but as the heating progresses the whole mass becomes a smooth plastic paste. Heating is continued for 20 minutes or half-an-hour; this really constitutes pasteurisation. Heating stops when the required consistence is reached, and the cheese is then poured direct into boxes with tin-foil linings or passed to the mechanical moulding-machines, which produce sectional pieces wrapped in tin-foil which are afterwards packed in the well-known circular boxes.

**The Ripening of Cheese.**—The analytical methods employed for cheese will not furnish much information without some knowledge of the changes taking place during ripening. As stated above, this process is essentially different in hard and soft cheese.

**Ripening of Hard Cheese.**—The process in this case is mainly chemical and enzymic. The part played by bacteria is practically limited to the production of lactic acid from the milk sugar, and as 99% of the bacterial flora are of the lactic acid type, the rest play probably an insignificant part, except perhaps in the production of flavour. For a comparison of the bacterial content and ripening process, see Harrison and Cornell (*Rev. gen. du Lait*, 3, 80 *et seq.*). The lactic acid as it is formed removes calcium from the curd and produces calcium lactate and acid calcium phosphate. The removal of the calcium, together with the action of the ferments of the rennet, produce during "cheddaring" a marked change in the nature of the protein which, from being almost insoluble in a 5% solution of sodium

chloride, becomes almost completely soluble. This soluble form then gradually passes into another insoluble form, and from this last arise, during the true ripening process, the variety of protein bodies found in the finished cheese. From the start of the true ripening process the amount of insoluble protein diminishes and the amount of water-soluble protein and its derivatives increases, so that, as Van Slyke has so ably demonstrated, "the amount of water-soluble protein and protein derived substances is a measure of the extent of the ripening process."

The production of these water-soluble and derived proteins is, in the light of our present knowledge, due to the rennet (perhaps the peptic constituent) in presence of lactic acid and its salts, the protein being gradually broken down into caseoses and peptones. Ripened cheese, however, contains large quantities of amino-acids and some ammonia and according to Boekhoult and de Vries these are produced by peptonising bacteria which gain entrance to the milk but which do not develop on account of the acid environment. They remain, however, distributed throughout the cheese and form foci for the secretion of proteoclastic enzymes. It has also been suggested that the lactic acid bacteria may eventually disintegrate in the cheese, and liberate such enzymes, but they do not produce any such effect by their ordinary activity. We find then, as a final result, that the nitrogen is present in very various forms, and the following table due to Van Slyke and Publowl illustrates this:

Age of cheese, months	Brine-soluble protein	Nitrogen expressed as percentage of nitrogen in form of:					
		Water-soluble proteins and derivatives	Para-nuclein	Caseoses	Peptones	Amino-acids	Ammonia
1½	20.18	21.44	2.06	3.15	3.84	0.88	1.56
3	27.26	30.98	4.45	4.56	4.65	14.36	2.45
6	27.55	36.15	3.57	4.92	4.22	19.06	3.52
9	24.14	43.45	4.02	4.59	3.56	26.53	4.74
12	19.04	44.75	3.52	4.16	3.95	28.38	5.41
18	12.65	47.25	3.40	3.88	2.57	30.46	6.62

The most noteworthy point in the above table is that, while the amount of amino-acids and ammonia steadily increases, the amount of intermediate products remains nearly stationary. This, however, has been fully explained by Van Dam (*Cent. f. Bakt.*, Abt. ii, 1910, 26,

189) as an outcome of experiments on the estimation of the acidity of cheese.

In certain cheeses it is of great importance to know the acidity of the cheese mass as it comes from the press, as on this acidity depends the course of the ripening. The extraction of the mass with either ether or acetone, as suggested by Boekhoult and de Vries, gives much too high results, as phosphoric acid and soluble phosphates appear in the extract, together with the lactic acid. Van Dam has therefore worked out a simple and easy adaptation of the conductivity method, which gives the true hydrogen-ion concentration, using not an extract of the cheese, but the actual cheese mass itself. In a similar way the action of the rennet has also been studied and the results seem to show clearly the course of events during ripening and explain the above apparent anomaly. He shows that in the case of Edam cheese, (though the facts are also true for other similar cheeses) the development of lactic acid in the press quickly reaches a maximum, while enzyme action which, at first was slight, then suddenly increases. He also shows that the digestion of the paracasein is then influenced by, and is proportionate to, the H-ion concentration, and that it only takes place through the agency of the chymosin of the rennet. Further, the solution of the cheese mass does not proceed immediately to the complete change of the paracasein but a state of equilibrium is brought about between it and its degradation products (peptones and caseoses). This equilibrium is then destroyed by the attack of bacteria or bacterial enzymes on the degradation products, with the result that the chymosin again dissolves true paracasein and so on. These findings explain the observed facts, viz.: that the formation of water-soluble nitrogenous substances is always rapid at first and then decreases, and that while amino-acids and ammonia steadily increase, the amount of intermediate products remains fairly constant during the ripening process. These results are quite in accord with the researches of Van Slyke and with our knowledge of the action of enzymes in general.

Whether good cheese can be made from milk which has been heated remains still a rather vexed question, but it would seem that if a proper "lactic" starter is used a fair quality cheese may result.

**Ripening of Soft Cheeses.**—In soft cheeses the ripening process is rapid and almost completely due to bacteria and moulds. A rapid production of lactic acid is essential (1) to protect the protein from

objectionable putrefactive processes, and (2) to produce a proper condition for the rapid development of moulds. A true ripening in soft cheese takes place from the periphery to the centre and the moulds as they push their mycelia into the substance of the cheese produce enzymes which break down the protein, while at the same time they use up the lactic acid already formed. Low temperatures are usually employed to favour the mould development, and "spearing" is resorted to if the growth is not sufficiently rapid. In such a cheese as Camembert the mould growth passes through the stages of a white felted mass until, on fructification, the well-known bluish-green colour appears, the mould finally breaking down and leaving the well-known reddish-brown coating.

For a full investigation into the action of fungi in the ripening of Camembert and Roquefort cheeses see Thorn, *Bulletin* 82, *Bureau of Animal Industry*, U. S. Dept. of Agriculture, Feb. 6, 1906.

M. Solari (*Anal. Ofic. Quim-Prov. Buenos Aires J.*, 1928, 1) has investigated very thoroughly the question of ripening in cheese. His observations, which extended over a period of nearly one year, and were carried out on several varieties of cheese, are amply illustrated by means of interesting tables. These figures of periodical analyses show clearly the fluctuation in composition as regards moisture, fat, albuminoids, amino compounds, acidity, etc. It was observed that the increase in these constituents is directly proportionate to the time and rate of the ripening process. Acidity gradually falls until about the fifth month and at this stage of the process decomposition of the fat begins. The organic acids begin to rise about the same time, and these advance rapidly from this point onwards.

**Flavour.**—The cause of "flavour" in cheese is still somewhat in dispute. In the case of hard cheeses of the "cheddar" type, the consensus of modern opinion seems in favour of the attribution of "flavour" to the amino-acids formed, but in soft cheeses the distinctive flavours are largely due to the various putrefactive bacteria, which produce small quantities of aromatic substances similar to those produced by them in game, etc., and also to the presence of large quantities of moulds which leave in the cheese their own distinctive flavour.

Before passing to the methods of analysis of cheese, the nature of some of the substances present must be considered.

**Fat.**—The fat of cheese made wholly from milk is ordinary butter fat. In hard cheeses it appears to undergo little change. There is little real evidence, if any, that fat arises from protein decomposition, but free fatty acids certainly occur in cheeses and the acid value of the fat obtained depends very much on the method of extraction.<sup>1</sup> This is well illustrated by some work of Koestler (*Milch Zent.*, 1908, 4, 111).

He shows that the character of the fat extracted by different methods varies slightly and solution of the curd in hot hydrochloric acid is not recommended by him. Extraction with ether, either with or without previous drying, is to be preferred, and he gives the following results with extraction methods using a margarine cheese. The ground-up cheese was extracted in one case with methylated ether and in the other with petroleum spirit, by shaking out 6 times in a separating funnel.

	Solvent	R. M. value	Sapon. value	Acidity c.c. N/10 KOH per 100 grm. of fat
I. Extraction of fresh cheese. . . .	Meth. ether.	3.02	198.9	161.0
	Pet. spirit.	2.12	199.0	110.9
II. Extraction after 24 hours drying at 50°.	Meth. ether.	2.70	201.6	142.3
	Pet. spirit.	2.80	199.7	119.0
III. Extraction after 10 days in vacuum desiccator.	Meth. ether.	3.51	199.3	197.3
	Pet. spirit.	2.64	198.2	95.4

It is easily seen that methylated ether extracts volatile acids which appear to be formed as the drying proceeds, or the extraction of which is facilitated by drying.

All the fat is not extracted in either way.

In Case I, 35% is not extracted by methylated ether and 71% not by petroleum spirit.

In Case II, 36% is not extracted by methylated ether and 29% not by petroleum spirit.

<sup>1</sup> Nierenstein (*Proc. Roy. Soc.*, 1911, B, 83, 301) states that old Cheddar cheese was found to contain free cholesterol, cadaverine, putrescine and aminovaleric acid, the amounts of these substances being sufficient to account for the increased weight of the ether extract frequently observed. The assumption that fat is produced from proteins during ripening does not seem to be justified.

Jensen, however, states that besides fatty acids derived from the milk fat, formic, acetic and propionic acids appear which are derived from the protein and milk sugar and that in certain cases butyric acid may be formed from milk sugar only.

Troili-Petersson and Gerda (abs. *Milch Zent.*, 1911, 7, 38) account for the fact that, while free fatty acids appear in cheese, no glycerol is ever detected, by showing that in certain cheeses (particularly Swedish *Herrensgutkäse*), there are present such organisms as will ferment glycerol, and they describe three strains of *Bact. glycerini*, a, b and c, which they have isolated from such cheese, and they also show that cheeses made from sterilised milk and inoculated with these organisms, together with lactic acid organisms and liquefying cocci, ripen normally and with a proper flavour.

Van Slyke also has drawn attention to the presence of small white specks in cheddar cheese cured at a low temperature. These specks appear to be calcium salts of a fatty acid, decomposition of the fat having probably taken place and so furnished the fatty acid.

The analytical figures obtained for the fat extracted from cheese must be taken with a little caution, as the effect of ripening is to alter the nature of the fat somewhat, but there is, of course, no difficulty about distinguishing a "filled" cheese from one made with true milk fat.

With regard to the percentage of fat in a cheese, the nature of the cheese must be borne in mind, as not all cheeses are made from whole milk.

Dr. A. J. Swaving<sup>1</sup> has published a collection of prescriptions concerning cheeses of which the following is a summary:

*United States of America.*—Cheese must not contain less than 50% of milk fat (calculated on the dry material). Skim and part-skim milk cheese must be so marked, and in the case of Edam, Roquefort and Camembert cheese quantity of contents of packages must be stated.

*Argentine.*—Addition of salicylic, boric acid and its salts is prohibited.

*Australia.*—All export cheese must be graded superfine, 1st grade, 2nd grade or 3rd grade, and no foreign matter, except rennet, salt or colouring matter deemed harmless by the Minister, may be added.

*Austria.*—Margarine cheese must contain 5% of sesame oil.

<sup>1</sup> *Collection of Legislative Prescriptions Concerning Cheese.* A. J. Swaving. The Hague: Hugo de Grootstratt. 1923.

Cream cheese must not contain less than	50% of fat in the dry substance
Whole milk cheese not less than	40% of fat in the dry substance
$\frac{3}{4}$ whole milk cheese not less than	30% of fat in the dry substance
$\frac{1}{2}$ whole milk cheese not less than	20% of fat in the dry substance
$\frac{1}{4}$ whole milk cheese not less than	10% of fat in the dry substance
Skim milk cheese not less than	10% of fat in the dry substance

Ratio of fat to protein on the dry substance must be about 1:0.83 for whole milk cheese, and 1:2 for  $\frac{1}{2}$  whole milk cheese. Gervais cheese must not contain less than 50% of fat, Camembert 30% and Camembert (fat) 40%, and in each case the water percentage must be 62.

*Belgium.*—No mineral substances, other than salt, and no anti-septics may be used.

*Brazil.*—

Cream cheese must contain	45% of milk fat in the dry substance
Whole milk cheese must contain	35% of milk fat in the dry substance
$\frac{1}{2}$ fat cheese must contain	25% of milk fat in the dry substance
Skim milk cheese less than	25% of milk fat in the dry substance

*Canada.*—Cheese must not contain less than 45% of milk fat in the dry substance, and cheese for export must be graded for flavour, texture, closeness, colour, and finish.

*Denmark.*—

	Minimum fat percentage con- tent (stamped on cheese)	Maximum water per- centage content
(A) Hard cheese		
Class I	45	50
Class II	30	54
Class III	20	57
Class IV	10	59
Class V. Skim milk		60
(B) Soft cheese (stamped on wrapper)		
Class VI	45	60
Class VII	30	60
Class VIII	20	60
Roquefort	50	52
Camembert	45	60
Gervais	50	60
Guarg		65

Emmenthaler Cheddar must be in Class I., Danish Swiss in Classes I. or II., Gouda, Edam, Tilsit, Steppecheese in Classes I., II., or III., and factory cheese in Classes III., IV. or V.

(Note.—These divisions are likely to be revised in the near future.)

*Esthonia.*—

Cream cheese must not contain less than	40% of fat in the dry substance
Whole milk cheese must not contain less than	30% of fat in the dry substance
$\frac{3}{4}$ fat cheese must not contain less than	25% of fat in the dry substance
$\frac{1}{2}$ fat cheese must not contain less than	20% of fat in the dry substance
$\frac{1}{4}$ fat cheese must not contain less than	10% of fat in the dry substance
Skim milk cheese less than	10% of fat in the dry substance

*Finland.*—Dairy factories have agreed that

Whole milk cheese should contain	45% of fat
$\frac{1}{2}$ fat cheese should contain	30% of fat
Skim milk cheese should contain	15% of fat

*France.*—Camembert cheese must contain 36% of fat by weight on the dry substance, and be exclusively made from cows' milk. Further regulations for cheese in general are probable.

*Germany.*—"Proposals for Regulations concerning Foodstuffs, 1913," suggest minimum fat percentages for cream cheese, 50; whole milk cheese, 40;  $\frac{3}{4}$  fat cheese, 30;  $\frac{1}{2}$  fat cheese, 20;  $\frac{1}{4}$  fat cheese, 10; skim milk cheese, less than 10 in the dry substance. Modifications may be expected.

*Ireland.*—Irish Free State manufacturers have agreed to 45% fat for whole milk cheese, and 25% for skim milk cheese in the dry substance.

*Italy.*—Cheese is considered to be made from margarine if the refractive index of the fat is above 48, and the Reichert-Meissl-Wollny number below 18. If the latter is between 18 and 24 the cheese is considered suspicious, and if above 24 as genuine. Margarine cheese may not contain added colouring matter.

*Netherlands.*—Manufacture of cheese is under strict Government control, and whole milk cheese must contain 45% of fat in the dry substance, and be stamped accordingly. Cheese made from partially skimmed milk must be stamped 40+, 30+, 20+, according to the proportion of fat contained:

*Norway.*—Cheese must be in one of the four following groups and be marked according to the fat content:

- I. Whole milk cheese with not less than 45% of fat in the dry substance
- II.  $\frac{1}{2}$  fat milk cheese with not less than 28% of fat in the dry substance
- III.  $\frac{1}{4}$  fat milk cheese with not less than 18% of fat in the dry substance
- IV. Skim milk cheese with less than 18% of fat in the dry substance

It is likely that the limits for groups II. and III. will be raised.



*Poland.*—

Whole milk cheese must contain	30-40% of fat
$\frac{1}{2}$ fat cheese must contain	20-30% of fat
Skim milk cheese must contain	10-20% of fat

and the maximum water content is 50% in the dry substance.

*Roumania.*—Cheese from part skimmed cows' milk must not contain more than 75% of water, and sheep cheese more than 55%, whilst mixed cheese from these milks must not have more than 70% of water. No other materials than lactic acid, rennet, salt, ferment or seasoning may be used in the manufacture of cheese.

*Switzerland.*—

Whole milk cheese must contain not less than	45% of fat in the dry substance
$\frac{3}{4}$ fat cheese must contain not less than	35% of fat in the dry substance
$\frac{1}{2}$ fat cheese must contain not less than	25% of fat in the dry substance
$\frac{1}{4}$ fat cheese must contain not less than	15% of fat in the dry substance
Skim milk cheese less than	15% of fat in the dry substance

The water content in Emmenthaler whole milk cheese must be between 26 and 36%, and for  $\frac{3}{4}$  fat Emmenthaler between 27 and 38%.

*Straits Settlements.*—

Whole milk cheese must not contain less than	50% of milk fat in the dry substance
Skim milk cheese must not contain less than	10% of milk fat in the dry substance
Cream cheese must not contain less than	60% of milk fat in the dry substance

No foreign fat, preservative other than salt, or colouring matter other than harmless vegetable colouring matter may be added.

*South Africa.*—Cheese must be graded into one of 3 grades for export according to flavour and aroma, quality (including body and texture), colour, salting, finish and general appearance.

*New Zealand.*—Quality of export cheese is rigorously controlled by Government, and standardised grading is being aimed at.

*Venezuela.*—Starch and gelatine may be added if stated, and all cheese not made with cows' milk must be marked in accordance with its origin, and the rind of artificial cheese must be coloured red. No cheese containing cheese-mites may be sold.

No special standards are in force in the following countries:—Algeria, British India, Bulgaria, Chili, Czecho-Slovakia, Grand Duchy of Luxemburg, Great Britain, Greece, Hungary, Lithuania, Livonia, Portugal, Serbia, Sweden, or Tunis.

**Mineral Salts.**—Apart from sodium chloride added during manufacture, the chief mineral constituents of cheese are calcium salts of

phosphoric acid. The precise state of combination of the calcium in milk is not fully understood; it may exist as dicalcium phosphate, or as calcium caseinate, or as both. Lactic acid as it develops removes the calcium from the curd and renders soluble any insoluble calcium phosphate producing calcium lactate and a soluble calcium phosphate. It has been shown that lactic acid removes calcium from casein in equivalent quantities, and also that casein can combine with free lactic acid. (*J. Hygiene*, 1907, 7, 216.)

There should not be in a normal cheese any free lactic acid, it should all be taken up by the cheese substances themselves. Failure in this results in a condition of cheese which is termed "short." This condition has been carefully investigated by Boekhout and de Vries (*Cent. f. Bakt.*, Abt. ii, 1909, 24, 122). The fault consists in the production of a granular hard white substance, either in specks or masses, in the cheese, and according to them is due to the formation of paracasein bilactate. They point out that in cheese there are three substances which can combine with the lactic acid, viz.: calcium phosphate, calcium paracaseinate, and the paracasein. From the first are formed calcium lactate and monocalcium phosphate, from the second calcium lactate and free paracasein, while addition compounds are formed between the acid and the paracasein which are supposed to be either monolactate or bilactate. It follows then that if either the cheese contains less than the usual quantity of lime salts, or there is an over-production of acid, there will be more acid to combine with the paracasein, and consequently in places the bilactate will be formed, and, as a result, the fault appears. Where, however, sufficient lime salts are present, the monolactate results and a normal consistence is obtained. Their experiments show that, on extracting normal and "short" cheeses with 5% salt solution, in which the bilactate is said to be insoluble, the content of bilactate in normal cheese varied from 1.3-2.4 c.c. 1/10 N. nitrogen, while in "short" cheese this figure rose to 15.8-18.4 c.c. They also show that the content of CaO in "short" cheese is less than in normal cheese. It follows then that milk exceptionally rich in lactose or the curd of which is poor in insoluble calcium salts is likely to produce this fault in cheese. Crystals of calcium phosphate are sometimes to be seen distributed in the cheese and may be recognised by heating the cheese with diluted alkali till the curd is slimy and then, after diluting with water, allowing to stand. The

crystal conglomerates settle out and may be washed with water and examined under the microscope.

With regard to the amount of sodium chloride present, this will naturally vary with the type of cheese, and may be in any percentage from 0.5–5.0%. It is interesting to note that in Edam cheese, which is packed in loose salt or pickled in brine, the salt distributes itself through the cheese during the ripening process. The following figures (Boekhoult and de Vries) show this very well:

CHEESE (Edam) DIRECT OUT OF BRINE

	Sodium chloride
Outer layers .....	13.3%
Middle layers .....	4.0%
Inner layers .....	0.4%
Same Cheese 4 weeks later	
Outer layers .....	5.0%
Middle layers .....	5.2%
Inner layers .....	4.4%

They state that the lowering of the salt percentage in the outer layer may be due to washing, which is done in order to remove excessive yeast and mould growth.

"*Process*" cheese is mostly made with the aid of emulsifiers, disodium phosphate being the cheapest of these. Williams (*J. Assoc. Off. Agric. Chem.*, 1927, 10) has determined the ratios of phosphoric acid and lime in a number of cheeses. The following table shows the results obtained:

Type of cheese	No. of samples	Ratio $P_2O_5$ : CaO		
		Maximum	Minimum	Mean
American Cheddar .....	28	1.210	1.023	1.094
Swiss .....	11	1.214	0.999	1.043
Brick .....	12	1.161	1.030	1.096
American Process .....	7	1.114	1.058	1.076 <sup>1</sup>
American Process .....	8	1.964	1.400	1.662 <sup>2</sup>

<sup>1</sup> No added disodium phosphate.

<sup>2</sup> Added disodium phosphate.

**Preservatives, Ripeners and Coatings.**—There have been numerous mineral substances found in cheese at various times, though they can scarcely be looked on as usual. *Lead chromate* appears to find entrance either from binding cloths impregnated with the chemical or else is used by mistake for colouring. *Lead dust* has also been found, but its mode of entrance is obscure. *Copper* certainly appears in certain cheeses, especially in the case of some Italian cheese, when copper vessels are used during manufacture. *Zinc sulphate* is said to be added as a "spice." Manfeld (*Abst. Z. Unters. Nahr. Genussm.*, 1911, 21, 428) describes a cheese preservative of the following composition—sodium formate 62%, sodium benzoate 10%, sodium chloride 28%; and also a "ripeners" consisting of sodium bicarbonate 70% and ground old cheese 30%.

Various materials have been suggested for hastening the ripening of cheese, such as ammonium carbonate and sodium or potassium carbonate. The following table gives details of three commercial preparations for the hastening of the ripening process. (Reiz, *Milch Zentralblatt.*, 1905, 1, 203):

Name	Maturin	Firmitas	Kasepreparat
Price	10 lb., 7.85 marks.	10 lb., 7 marks.	10 litres, 7 marks.
Accelerating power	Ripening in half the time.	Marketable cheese in 10-12 days.	
Quantity employed	$\frac{1}{4}$ – $\frac{1}{2}$ lb. per 100 lb. of curd.	1–2 lb. per 100 lb. curd.	
Appearance	White powder.	Yellowish powder.	Reddish liquid.
Smell	None.	Like old cheese.	None.
Sp. gr. at 15°			1.076
Moisture	1.05%	11.85%	
NaHCO <sub>3</sub>	48.72%	57.7%	8.40 grm. per 100 c.c.
Na <sub>2</sub> CO <sub>3</sub>	Trace	None.	1.01 grm. per 100 c.c.
NaCl	50.25%	3.55%	Trace.

**Coatings.**—The question of the coating of cheeses with heavy artificial rinds, has arisen particularly in connection with Gorgonzola cheese, in which case a thin coating containing a large proportion of barytes has been sometimes employed. As this adds very materially to the weight, it has been looked on as a fraudulent proceeding and legal action has been taken to put a stop to it. (See Hinks, *Analyst*, 1911, 36, 61.)

The report of a commission held in 1900 in Italy on the question is against the use of baryta in the coating, and their recommendations are:

(1) That cheeses which are exported in the cooler months of the year scarcely need a coating.

(2) That for cheeses which will not stand the heat of the summer without losing their shape and taste, a light coating should be employed which should not be of an objectionable nature and should be free from barium sulphate.

It has become an established practice to coat such cheeses as Cheddar intended for export with a thin layer of paraffin wax. This procedure greatly improves the keeping quality of the cheese and is quite legitimate.

**Alkaloids.**—Adametz and Chrsaszcz (*Oesterreich Molkerei Zeit.*, 1905, No. 3-5) have shown that an alkaloid called by them *tyrothrixin* occurs in Emmentaler cheese in which *B. nobilis* has been used in the ripening process. This alkaloid has given the following chemical properties:

A snow-white crystalline substance of very characteristic pungent odour, easily soluble in alcohol, ether and dilute acids, soluble with difficulty in water at ordinary temperatures and insoluble in sodium or potassium hydroxide solution.

Phosphomolybdic acid gives a voluminous canary-yellow precipitate.

Phosphotungstic acid a white precipitate soluble in excess.

Potassium mercury iodide solution produces a sulphur-yellow precipitate in long needles.

Gold chloride gives a citron-yellow precipitate which deposits metallic gold in about 15 minutes.

Platinum chloride and tannin produce no precipitate.

		Water, %	Fat, %	Protein, %	Ash, <sup>1</sup> %
Green cheeses.....	Average.	36.84	33.83	23.72	5.61 <sup>1</sup>
	Maximum.	43.89	36.79	26.11	7.02 <sup>1</sup>
	Minimum.	32.69	30.00	20.80	3.12 <sup>1</sup>
Seven weeks old.....	Average.	36.06	34.43	24.45	3.61
	Maximum.	41.15	45.36	28.72	5.29
	Minimum.	32.23	23.27	18.45	1.81
Five months old.....	Average.	34.01	36.81	25.69	3.50 <sup>1</sup>
	Maximum.	38.10	44.33	30.09	4.59 <sup>1</sup>
	Minimum.	29.85	27.22	21.53	2.72 <sup>1</sup>

<sup>1</sup> Includes traces of milk sugar.

The best collection of analyses and descriptions of manufacture of the world's cheeses will be found in *Bulletin 146, Bureau of Animal Industry*, U. S. Dept. of Agriculture, 1911.

The table on p. 194 shows analyses of American Cheddar cheese made by Van Slyke, and the following analyses of English Cheddar are by Voelcker:

	Water, %	Fat, %	Protein, %	Lactose and lactic acid, %	Ash, %
Average.....	35.16	30.45	27.80	3.16	3.42
Maximum.....	39.43	41.58	32.37	6.80	4.31
Minimum.....	30.32	23.21	23.28	0.22	2.06

Cheshire cheese is not generally regarded as genuine if the proportion of fat, calculated upon the dry sample, is less than 45%.

The following analyses of Gorgonzola cheese are by Musso:

	Water, %	Fat, %	Protein, %	Lactose lactic acid, etc., %	Ash, %
Average.....	37.30	34.67	25.16	1.62	3.82
Maximum.....	47.10	39.32	28.51	2.00	4.63
Minimum.....	29.82	29.00	20.33	0.91	3.13

As a result of a number of analyses of Emmentaler cheese made in 1909 and 1910, Koestler (*Milch Zentralblatt*, 1910, 6, 289) gives the following mean values:

No. of analyses	Water, %	Total solids, %	Fat		Protein		Fat Protein
			in fresh cheese, %	in dry solids, %	in fresh cheese, %	in dry solids, %	
37 10	33.63	66.37	32.20	48.53	27.49	41.42	1.17
	33.09	66.91	31.44	47.00	27.84	41.64	1.13

He is of the opinion that the fat should not fall below 45% in the dry solids. The paper also contains a very interesting series of analyses made from sections of a piece of cheese cut right through a complete cheese. The results show that great uniformity of composition exists in such a boring.

The following analyses of Dutch cheese have been made by Paraschtschuk, 1909:

Water	Ash, %	Fat, %	Fat in dry solids, %	Lactic acid, %	Protein
33.57	5.45	22.70	34.31	1.29	36.90
35.13	6.02	27.78	42.82	1.17	29.90
32.57	5.70	32.20	47.80	1.31	28.17
32.22	5.65	34.04	50.22	0.95	27.14

The Reichert-Meissl value of the fat varied from 26.74-29.80, and the acid value from 1.9-9.7.

The following analyses of Portuguese cheeses are given by Cordoso and Mastbaum (*Chem. Zeit.*, 1904, 28, 989).

For curdling the milk, in many cases, the heads of thistles are ground with water in mortars and the strained liquid added to the milk.

Milk of	Name of district	Water, %	Protein, %	Fat, %	Sugar, %	Ash, %	NaCl, %	Acidity as lactic acid, %	Refraction number	Sap. value
Sheep.....	Forminhao.	45.79	24.13	21.59	3.70	4.79	1.92	1.18	51.5	.....
Sheep.....	Alcanis.	43.96	19.87	22.30	5.43	8.44	4.63	0.79	51.0	219.0
Sheep.....	Azeitao.	42.54	21.97	25.27	3.82	6.40	3.20	1.33	50.5	.....
Sheep and goat	Castello branco.	42.83	21.21	28.47	0.58	6.91	4.94	.....	54.5	225.0
Sheep and goat	Aldeias.	38.26	32.10	19.30	4.43	5.91	2.10	0.73	54.0	239.0
Goat.....	Tinalhas.	45.55	20.19	23.10	6.78	4.38	1.88	0.96	54.5	242.0
Cow.....	Gouvein.	49.51	17.83	26.10	2.81	3.23	0.94	0.34	50.5	239.0
Cow.....	Lissabou.	54.37	13.63	25.84	2.06	3.20	2.04	0.57	55.0	250.0
Sheep, goat and cow.	Cardiga.	28.39	27.32	34.37	5.00	4.92	2.05	1.83	52.5	238.0

The following are analyses of Camembert cheeses given by Bittenberg and Guth (*Z. Unters. Nahr. Genussm.*, 1907, **14**, 677 and 1908, **15**, 416):

Kind of cheese	Description	Water (1) %	Fat free dry matter (2) %	Fat (3) %	Ratio (3):(2)
Magerkäse.....	Extra fine, German	56.36	35.16	8.48	1:4.15
Halbfetterkäse....	Baby	47.22	39.56	13.22	1:2.99
Halbfetterkäse....	Petit	54.75	30.06	15.19	1:1.98
Fetterkäse.....	Gourmand	44.36	36.79	21.85	1:1.69
Fetterkäse.....	Demilunaise	57.15	25.26	17.59	1:1.43
Fetterkäse.....	Petit, G. U.	53.11	27.57	19.32	1:1.43
Vollfetterkäse....	Vrai Lisieux	52.47	25.17	22.36	1:1.13
Vollfetterkäse....	Le Laurier	57.35	21.48	21.17	1:1.01
Vollfetterkäse....	Edelweiss	51.37	21.44	27.19	1:0.79

The following estimations of fat and total solids in various cheeses are due to Vieth (*Jahresb. d. Milch Instituts zu Hameln*, 1909, 32):

Cheese	Total solids, %	Fat, %	Fat in total solids, %
Tilsiter.....	48.58	10.38	21
	56.22	14.21	25
	57.20	27.90	49
	58.20	28.50	49
	61.20	25.20	41
Chester.....	50.90	14.30	28
Brie.....	38.50	18.76	49
	47.05	23.80	51
Ramadour.....	46.85	20.86	45
	48.90	13.32	27
	43.00	9.50	22
Limburger.....	48.45	21.30	44
	38.77	7.83	20
	43.12	7.40	17
Kaiserkäse.....	40.20	20.85	52
Frühstückkäse.....	39.00	11.65	30
Backsteinkäse.....	45.16	6.22	14

The following analyses of the principal cheeses used in France are by Liudet, Ammaun and Brugière:



Cheese	Water, %	Fat, %	Pro- tein, %	Ash		Soluble nitrogen in % of total nitrogen
				Insolu- ble, %	Solu- ble, %	
Troyes.....	58.7	18.6	14.6	1.1	3.7	70.8
Mont D'or.....	58.7	9.7	25.3	2.4	1.9	39.8
Coulommiers Double Cream.....	57.8	25.0	13.0	0.5	3.6	44.4
Petit Suisse.....	54.6	35.0	7.3	0.5	0.1	3.2
Bondon.....	54.3	23.0	16.1	0.7	4.3	32.9
Camembert.....	53.8	22.0	17.1	1.2	3.2	86.1
Brie.....	53.5	22.5	18.0	0.8	3.2	58.1
Reblochon.....	53.2	20.5	10.3	1.9	1.8	27.9
Livarot.....	52.2	15.0	25.0	1.5	2.9	55.9
Pont l'Eveque.....	51.0	23.1	17.8	2.1	1.9	43.9
Demi-sel.....	49.6	34.0	11.8	0.6	2.4	12.2
Hollande.....	42.6	20.0	23.0	2.3	3.2	22.3
Gorgonzola.....	41.5	29.0	19.7	2.2	2.6	27.2
Cantal.....	40.9	29.3	20.5	2.2	2.6	46.0
Marolles.....	40.3	33.5	20.2	1.2	3.3	59.4
Port Salut.....	38.1	24.5	24.8	3.1	2.2	20.2
Roquefort.....	36.9	29.5	20.5	1.9	5.1	47.5
Gruyère.....	35.7	28.0	28.9	3.1	0.4	22.9
Parmesan.....	34.0	23.0	35.0	3.5	1.7	21.7

The following analyses of Norwegian cheeses are given by Hals (*Z. Unters. Nahr. Genussm.*, 1909, 17, 673):

They are average results.

Cheese	Milk	Water, %	Ash, %	Fat, %	Protein, % N <sub>2</sub> × 6.25	Lactose, %
"Halbfetter"....	Goat.	23.25	6.31	14.65	5.05	50.74
"Fetter".....	Goat and cow.	12.74	4.52	30.82	10.21	41.71
"Magerer".....	Sour cow's milk.	22.74	8.74	0.73	11.47	52.47

The cheese is actually made by boiling down whey in copper or iron vessels to the required consistence and then running into wooden tubs. It is practically not ripened at all.

*Kräuter Käse* is usually made from butter milk mixed with skim milk and with the addition of *Mellilotus caerulea*. It is often in the form of a powder. The following analyses have been published by Buttenberg and Koenig (*Z. Unters. Nahr. Genussm.*, 1909, 18, 413):

	Total solids, %	Fat, %
Powder form from skim milk.....	76.53	5.10
Shaped cheese from skim milk.....	36.20-47.59	3.4-7.8
Shaped cheese from richer milk.....	38.88-55.34	4.68-12.20

The cheese is also made with an addition of coconut oil, when the total fat content rises as high as 38-39% and the saponification value of the fat varies from 241.91-256.99 and the R. M. value from 8.36-14.25.

The following are analyses of Margarine cheeses (Koestler, 1908):

No.	Water, %	Total solids, %	Fat in fresh cheese, %	Fat in dry solids, %	R. M. No.	Sap. No.	Acidity in c.c. N/10.KOH per 100 grm. fat
1					3.7	194.1	219
2					8.1	194.2	
3					6.7	194.3	
4	36.13	62.87	9.89	15.73	6.6	190.9	
5	42.78	57.22	11.66	20.28	3.9	200.7	197
6	44.88	55.12	12.03	21.82	3.7	191.1	361
7	38.44	61.56	11.12	18.06	4.3	195.0	204
8	38.08	61.92	8.06	13.02	1.9	201.6	166
9	36.64	63.36	9.28	14.64	2.7	198.9	136

The fat was extracted from the fresh cheese with methylated ether. Schaeffer gives the following variations in the fat content of cheeses:

Camembert.....	28.03-51.17 (17 samples)
Brie.....	38.15-50.85 (6 samples)
Neufchâtel.....	43.28-52.54 (7 samples)
Gervais.....	64.07-64.38 (2 samples)
Tilsiter.....	23.07-48.07 (9 samples)
Russian Steppenkäse.....	44.00-48.18 (2 samples)
Limburger.....	12.14-47.68 (28 samples)
Romadour.....	22.56-45.83 (8 samples)

### TIN IN WRAPPED CHEESE

The presence of tin in rindless cheeses which have been packed in tinfoil has been reported by Elten (*Chem. Ztg.* 1929, **53**, 586), the foil consisting of 96 to 98% tin and 2 to 4 % antimony. C. H. Manley (*Analyst*, 1930, **55**, 191) reports that a similar tinfoil was proved to contain 96.8% of tin and 3.2% of antimony, with a

trace of iron as impurity. The cheese around which it was wrapped contained: tin, 160 parts per million (1.12 grains per lb.) and antimony, 17 parts per million (0.12 grains per lb.). In two other specimens the tin amounted to 15 and 160 parts per million, and discolouration of the cheese was very pronounced. In the latter of these two cheeses the wrapping consisted of pure tinfoil.

### The Analysis of Cheese

A representative sample must be obtained. In the case of large cheeses of the hard variety 2 or 3 borings with a large cork borer should be made. The part containing the rind, and at least half an inch within it is cut off and the remainder cut into fine shavings with a grating machine or bread grater.

**Water.**—2 to 5 gm. are weighed out into a large flat weighing bottle or platinum dish and dried in a water-bath to constant weight.

More accurate results are obtained by drying for 2 days in a vacuum desiccator and then for 8-10 hours in the water bath.

**Ash.**—This may be estimated by burning off a few gm. of the cheese in a platinum dish, first over a burner till the fat is destroyed, and finally at a very low red heat in a muffle.

It is, however, preferable to extract the bulk of the fat with warm ether once or twice, pouring the ether off through a small filter paper which is afterwards added to the cheese in the dish and burnt. When an estimation of phosphates and calcium is required, incineration is best carried out in a covered crucible after the addition of an equal amount of a mixture of 1 part of potassium nitrate and 2 parts of sodium carbonate; otherwise a considerable loss in phosphoric acid will occur. An alternative method is to boil the cheese with 30 c.c. of strong nitric acid and 5 c.c. of strong hydrochloric acid in a flask (fitted with a cooling tube in the neck) until clear. It is then evaporated to dryness, taken up with water, neutralised with strong potassium hydroxide solution, evaporated and incinerated.

**Fat.**—This may be roughly estimated by the Gerber or Babcock methods. In the former case 0.5 to 1.0 gm. of the cheese is rubbed down in a small beaker with a little warm water, and a drop or two of ammonia, and washed into the Gerber bottle with so much water as will bring the total quantity used up to 10 c.c. The test is then finished as with cream.

For the Babcock method, Lythgoe directs that about 6 gramm. should be placed in a beaker and 10 c.c. of boiling water added, an emulsion being made by rubbing with a rod after the addition of a few drops of strong ammonia. About half the usual 17.6 c.c. of sulphuric acid is added to the beaker, and after stirring, the contents are poured into a Babcock cream bottle, the beaker being washed out with the remainder of the acid. The test is carried out in the usual manner, the reading being multiplied by 18 and divided by the weight of cheese taken.

The Gottlieb method is quite applicable to cheese, but will give only the neutral fat present. As a certain amount of free fatty acids is usually present, the acid method is preferable.

If the fat is to be extracted for further examination, a large quantity of the cheese is dissolved in boiling hydrochloric acid as above described, and the fat is either extracted with ether or separated by rotation and clarified by passing through dry filter paper.

In any case, as noted above, the nature of the fat extracted will vary somewhat according to the method employed.

Schaffer and Fellenberg (1910) have estimated the constants for the fat extracted from various cheeses by the hydrochloric acid method and with petroleum and methylated ether; and also with these ethers after close admixture of the cheese with calcium chloride. The highest Reichert-Meissl values were obtained by this last method.

The following are some figures given by them for whole and margarine cheeses:

Cheese	Refractometer No. 40°	R. M. No.	Amount of fat employed for R. M. No.	Sap. No.	Iod. value
Margarine . . . . .	47.12	8.94	2.0	223.6	43.5
Whole . . . . .	46.8	20.0	2.0	223.4	42.0
Margarine . . . . .	46.0	5.0	2.5	201.1	45.0
Whole . . . . .	42.2	27.2	5.0	222.6	41.1
Margarine . . . . .	41.2	11.25	2.0	215.3	38.6

In all cases the fat was extracted after mixing the cheese with calcium chloride.

The fat is examined by the methods described under butter-fat (Vol. II, p. 359 *et. seq.*).

The question of the accurate determination of fat has been fully investigated by several workers, particularly by Utz, (*Milch Zentralbl.*, 1913 42, 457) who considers that the best methods are (1) the Ratzliff-Schmid-Bondzynski and (2) the Polenske.

(1) *The Ratzliff-Schmid-Bondzynski Method.*—From 3–5 grm. of cheese are placed in a flask with 10 c.c. of hydrochloric acid (sp. gr. 1.125) and boiled gently over a small flame 8–10 minutes. The solution is cooled and poured into a Gottlieb tube, 25 c.c. of methylated ether and petroleum spirit respectively added, in that order, mixing the solution gently by inversion after each addition. The ethereal layer is allowed to separate for 6 hours, pipetted off into a weighed flask and the acid liquor extracted once more with the same volume of ethers. The ether in the weighed flask is then distilled off and the fat dried to constant weight.

(2) *The Polenske Method.*—From 1–1.5 grm. of cheese are placed in a conical flask with 10 c.c. of water and 5 c.c. sulphuric acid (sp. gr. 1.81–1.84) and the mixture heated over a small flame, being allowed to boil gently for 2 minutes, a cooling tube being preferably arranged in the mouth of the flask. 35 c.c. of water are then added, the solution cooled, and 50 c.c. of methylated ether (at 18°) run in and the mixture gently shaken. 50 c.c. of petroleum spirit (at 18°) are added and the mixture again gently shaken for 1 minute, and then cooled in water at 18°. After 15–20 minutes 49.5 c.c. of the ether are pipetted off and passed through a cotton wool filter into a small weighed flask. The cotton wool is washed two or three times with a little ether, the ether then distilled off and the fat dried and weighed.

The final proposals (April 27, 1913) of the *Committee of the Fédération Internationale de Lâiterie* for the unification of analytical methods for cheese are as follows:

(1) **Estimation of Water.**—2–3 grm. of the mixed sample of cheese are quickly weighed into a suitable flat nickel or platinum dish, containing either ignited coarse quartz powder or sea sand purified by hydrochloric acid, and a glass stirrer, and the cheese is mixed with the quartz powder or sea sand as equally as possible. The dish is then heated in an oven at 105–110°.

After about  $1\frac{1}{2}$  hours the weight is determined and weighings are made at the expiration of succeeding half hours till the weight ceases to diminish. The weight of the cheese residue is taken as that of the *dry substance*, the loss in weight as the water of the cheese.

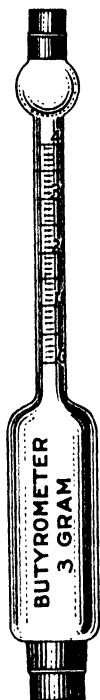


FIG. 7.—Butyrometer, Gerber-Van Gulik. ( $\frac{1}{2}$  actual size.)

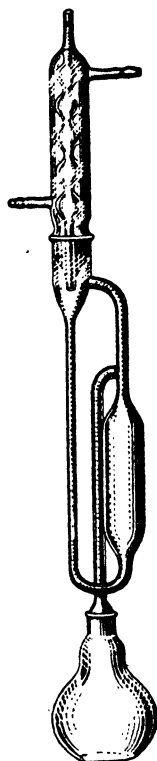


FIG. 8.—Smetham's flask. ( $\frac{1}{8}$  actual size.)

(2) **Estimation of Fat.**—(A) *By Gerber's method (with modified cheese butyrometer of Van Gulik) for 3 gm. of cheese.*

(A) *Approximate Method.*—3 gm. of cheese are introduced with the aid of a funnel into the widened part of the cheese butyrometer (Fig. 7) which has previously been half filled with sulphuric acid (sp. gr. 1.50). The weighing of very soft cheese is done in a beaker.

The butyrometer is then warmed in a water-bath at  $65-70^{\circ}$ , and the casein dissolved, with shaking and swinging of the butyrometer at

intervals. When no more particles of cheese are visible the butyrometer is left in the water-bath for a short time, with swinging at intervals, and then after 1 c.c. of amyl alcohol has been added, it is filled with sulphuric acid nearly up to the mark 35. The contents are mixed with care, not swinging (or shaking) the butyrometer more than necessary to ensure complete mixing, then left for about 5 minutes in the water-bath and whirled at a speed of 1,000 rotations per minute. The diameter of the disc-plate of the Gerber centrifugal machine must be at least 47 cm. (the machine should be warmed when the temperature of the room is low). Finally the butyrometer is placed in a water-bath for 5 minutes at 65°, and the height of the fatty layer is read at 65°.

(B) *Hydrochloric Acid Method.—Final Method.*—From 1–5 grm.<sup>1</sup> of rasped cheese are gently boiled with 20 c.c. of hydrochloric acid (sp. gr. 1.13) in a small flask, which is shaken until all small particles have disappeared.

Then either: (1) *Smetham's percolation method* or (2) the *modified Schmid-Bondzynski method* is applied.

(1) *Smetham's Percolation Method.*—The hydrochloric acid solution, when cool, is carefully introduced into the flask of a Smetham's extractor (Fig. 8), as modified for the estimation of fat in milk, and the flask is filled with ether until it flows over.

After extraction for 2 hours the ether is evaporated and the fat dried for half an hour in the oven at 102°. Drying is continued to constant weight.

For control, a second flask can be connected to the extractor and treated as above.

(2) *Modified Schmid-Bondzynski Method.*—The hydrochloric acid solution is carefully introduced into a 100 c.c. cylinder and, when cool, ether is added until the total volume is about 50 c.c. The cylinder is closed with a damped cork, well shaken, and left for at least 2 hours for the two liquids to separate completely. The ether-fat solution is then drawn off as completely as possible (to at least 1 c.c.) into a weighed flask. A fresh quantity of 50 c.c. of ether is introduced into the cylinder and the contents swung several times. After standing 1 hour the ether is again drawn off as completely as possible and added to the first ether-fat solution in the weighed flask.

<sup>1</sup> In applying this method it is advisable to use, within these limits, a smaller quantity of fat cheese and a larger one of skim milk cheese.

The ether is then distilled off and the fat dried for half an hour in the oven at  $102^{\circ}$ .

Drying is continued to constant weight.

**Lactic Acid.**—This is usually estimated by rubbing 10 grm. of the cheese with warm water at  $40-50^{\circ}$  and making up the volume to 105 c.c. This is filtered clear and 25 c.c. of the filtrate titrated with  $N/10$  sodium hydroxide to phenolphthalein.

As stated above, however, accurate estimations of the lactic acid can only be carried out by the conductivity method, and the methods of extraction which have been employed are also open to objection.

**Total Protein.**—From 1-2 grm. are treated by the Kjeldahl-Gunning method in the usual way. The result, however, is not accurate as some of the protein is in the form of amino-acids and ammonia; the usual factor will, therefore, over-estimate the protein.

**Examination of the Nitrogenous Compounds.**—This will not fall under the heading of ordinary analyses, but as the examination may have to be made in certain cases, the following methods are appended. The most complete method is that of Van Slyke:

Twenty-five grams of the sample are ground in a mortar with sufficient quartz sand. The mixture is washed into a flask with about 100 c.c. of water at  $50^{\circ}$  and is maintained at this temperature for 30 minutes, with frequent shaking. The liquid is then decanted through a cotton wool filter into a 500 c.c. flask, and the residue exhausted in a similar way with further quantities of 100 c.c. of water till nearly 500 c.c. are obtained. The flask is cooled to room temperature and made up to the mark, the layer of fat on the surface being neglected.

This stock solution is used for the following estimations:

**Water Soluble Nitrogen.**—50 c.c. (= 2.5 grm. of cheese) of the solution are treated by the Kjeldahl-Gunning method.

**Nitrogen as Paranuclein.**—To 100 c.c. of the stock solution are added 5 c.c. of 1% hydrochloric acid, and the mixture kept at  $50-55^{\circ}$  till a clear supernatant liquid appears. The precipitate is filtered off, washed with water, and the nitrogen estimated in it.

**Nitrogen as Coagulable Protein.**—The filtrate from the last is neutralised with dilute potassium hydroxide and heated in boiling water, till any coagulation which may occur is complete. The precipitate is filtered off, washed, and the nitrogen estimated.

**Nitrogen as Caseoses.**—The filtrate from the last is treated with 1 c.c. of 50% sulphuric acid saturated with C. P. zinc sulphate, and



warmed to 70° till precipitation is complete. The mixture is cooled and filtered, the precipitate being washed with the saturated acid solution of zinc sulphate and the nitrogen estimated in it.

**Nitrogen as Amines and Peptones.**—100 c.c. of the stock solution are placed in a 250 c.c. flask. To this are added 1 grm. of sodium chloride and a 12% solution of tannin until no further precipitation takes place. The flask is filled to the mark, the contents mixed and poured through a dry filter, and the nitrogen estimated in 50 c.c. of the filtrate. This, less the nitrogen due to ammonia, in a similar quantity, gives the amino nitrogen. Total nitrogen in stock solution—(nitrogen as paranuclein, coagulable protein, caseoses, amines and ammonia) = nitrogen as peptones.

**Nitrogen as Ammonia.**—100 c.c. of the filtrate from the tannin precipitation are distilled into standard acid, and the ammonia passing over is estimated.

**Nitrogen as Paracasein Lactate.**—The residue of the original cheese insoluble in water is treated with several quantities of 5% solution of sodium chloride until 500 c.c. are obtained. The nitrogen is estimated in 50 c.c.

A rather less complicated method of controlling ripening is given by Sanfelici (*Annuario R. Stazione Sperimentale di Caseificio di Lodi*, 1907, 65).

Twenty grm. of finely ground cheese are rubbed up with sufficient warm water and placed in a 500 c.c. flask, the mixture made up to the mark and allowed to stand for a short time. Three layers form, an upper fat layer, a middle watery layer, and a lower layer consisting of the undissolved substances. The watery layer is drawn off and filtered several times. In 50 c.c. the total quantity of soluble nitrogen is estimated; in another 50 c.c. the albumoses and peptones are precipitated with phosphotungstic acid, and the filtrate made up to 100 c.c. In this the amino-acid nitrogen is estimated; the precipitate itself is washed with dilute sulphuric acid, filtered, the residue dried, and the nitrogen estimated. Another 50 c.c. of the original filtrate are diluted with water and distilled with magnesium oxide, and the ammonia estimated. The nitrogen in this last is subtracted from the nitrogen obtained in the phosphotungstic acid filtrate.

The volatile fatty acids of cheese are best estimated by mixing 100 grm. of the finely divided cheese with 200 c.c. of water and 1.5–2.0 c.c. of conc. sulphuric acid and steam distilling till 1,000 c.c. have

passed over. The filtrate is then titrated with  $N/10$  barium hydroxide, and, if desired, the mean molecular weight can then be found.

**Determination of Tin in Cheese.**—The following is a modification by Monier-Williams of a method due to Owe (*Z. Unters. Nahr. Genussm.*, 1926, **51**, 214). An appropriate quantity of the cheese (15 to 100 grm. according to the amount of tin expected to be present) is ashed at as low a temperature as possible, preferably in a muffle furnace. The ash is dissolved in about 25 c.c. of concentrated hydrochloric acid, and 60 c.c. of water are added. The liquid is transferred to a 300 c.c. conical flask, fitted with a two-holed stopper and glass tubing so that a current of carbon dioxide can be passed through the contents. 0.4 grm. of aluminium turnings is added and allowed to react in the cold for 5 to 10 minutes, carbon dioxide being passed. The solution is warmed, without boiling, until the aluminium has dissolved, and is then boiled for 5 minutes until all particles of tin are dissolved, after which the flask is cooled, carbon dioxide still being passed. 25 c.c. of  $N/50$  iodine are added, and the solution diluted with well-boiled cold water to 200 c.c. The excess of iodine is titrated with  $N/50$  sodium thiosulphate, and the proportion of tin calculated by means of the relation

$$1 \text{ c.c. of } N/50 \text{ iodine} = 0.00117 \text{ grm. of tin.}$$

Small quantities of iron do not appear to affect the result, but copper (if present in amounts of the same order as that of the tin) introduces a positive error. If appreciable quantities of copper be suspected, both copper and tin must be determined, preferably by separation of the sulphides in the manner usually employed for foodstuffs.

**Examination of Cheese for the Presence of Iron and Copper.**—Cheese often contains traces of both iron and copper. Iron, particularly, has a prejudicial effect as, if present in as much as 0.0005% (calc. as  $\text{Fe}_2\text{O}_3$ ), the marketable condition of the cheese is prejudiced. Copper appears much less frequently and not till 0.001% (calc. as  $\text{CuO}$ ) is reached, is a prejudicial effect on colour noted. The following method of estimating iron in such traces as occur in cheese has been worked out by Schaeffer:

Twenty grm. of cheese are kneaded in a porcelain dish with as much ammonia as is necessary to dissolve the whole mass. From 1–2 c.c. are usually sufficient, but the cheese must be reduced to a transparent mass and the smell of ammonia must be apparent.

Then add 5 drops of yellow ammonium sulphide, knead the whole again, place the mass on a piece of opal glass and after 10 minutes compare the colour with standards made by adding known amounts (calc. as 0.002%  $F_2O_3$ ) of ferric chloride solution to iron-free cheese.

The detection of small quantities of copper is not easy. About 20 grm. of the cheese are grated up and heated gently, with occasional stirring, in a porcelain basin with 5 c.c. of strong sulphuric acid (free from copper) for 1 to 2 hours till all spirting has ceased. The dish is then heated strongly for 1 to 3 hours till the mass is thoroughly charred. The char is broken up and again heated till all carbon is burnt off, and the residue is dissolved by repeated extraction with hot dilute hydrochloric acid. This solution is made slightly alkaline with ammonia and then faintly acidified with dilute hydrochloric acid. Hydrogen sulphide is passed for 1 hour, the liquid then being allowed to stand covered for 12 hours, and the gas again passed for  $\frac{1}{2}$  to 1 hour. The copper sulphide is filtered off and dissolved in hot dilute nitric acid, and ammonia is then added in excess, and the solution evaporated or diluted to 15 c.c., filtered if necessary, and made up to 20 c.c. To this are added 1 c.c. of strong acetic acid and 1 c.c. freshly made solution of potassium ferrocyanide, and the colour compared with a standard solution of copper sulphate, portions of which are made up to 20 c.c. with 10% ammonium nitrate.

**Microscopical Examination of Cheese.**—For examining bacteria *in situ* in cheese, the simple method of Rodella (*Cent. f. Bakt.*, Abt. ii, 1906, 15, 143) is excellent. The cheese is cut into small cubes, and each cube is laid between 2 slightly warmed cover slides and gentle pressure exerted. An impression specimen of the surface of the cube is thus obtained, and after washing with chloroform and alcohol to remove fat, the film is stained with very dilute carbol-thionin or methylene blue. (This method is probably due to Johan-Olsen.)

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# MEAT AND MEAT PRODUCTS

BY C. ROBERT MOULTON

## Definitions of Meats

Meat and meat products are defined in an order of the Secretary of Agriculture of the United States as follows:

### (a) MEATS

1. **Flesh** is any clean, sound, edible part of the striated muscle of an animal. The term "animal," as herein used, indicates a mammal, a fowl, a fish, a crustacean, a mollusc, or any other animal used as a source of food.

2. **Meat** is the properly dressed flesh derived from cattle, from swine, from sheep, or from goats, sufficiently mature and in good health at the time of slaughter, but is restricted to that part of the striated muscle which is skeletal or that which is found in the tongue, in the diaphragm, in the heart, or in the œsophagus, and does not include that found in the lips, in the snout or in the ears; with or without the accompanying and overlying fat and the portions of bone, skin, sinew, nerve, and blood vessels, which normally accompany the flesh and which may not have been separated from it in the process of dressing it for sale.

The term "meat," when used in a qualified form, as for example, "horse-meat," "reindeer meat," "crab meat," etc., is then, and then only, properly applied to the corresponding portions of animals other than cattle, swine, sheep, and goats.

3. **Fresh** meat is meat which has undergone no substantial change in character since the time of slaughter.

4. **Beef** is meat derived from cattle nearly one year of age, or older.

5. **Veal** is meat derived from young cattle one year or less of age.

Minimum limits governing the age or the weight or both of these have been fixed by certain States and Municipalities in the case of calves to be slaughtered for meat.

6. **Mutton** is meat derived from sheep nearly one year of age, or older.

7. **Lamb** is meat derived from young sheep one year or less of age. Minimum limits governing the age or the weight or both of these have been fixed by certain States and Municipalities in the case of lambs to be slaughtered for meat.

8. **Pork** is meat derived from swine.

9. **Venison** is flesh derived from deer.

#### (b) MEAT BY-PRODUCTS

1. **Meat by-products** are any clean, sound, and properly dressed edible parts, other than meat, which have been derived from one or more carcasses of cattle, of swine, of sheep, or of goats, sufficiently mature and in good health at the time of slaughter.

#### (c) PREPARED MEATS

1. **Prepared meat** is the clean, sound product obtained by subjecting meat to a process of comminuting, of drying, of curing, of smoking, of cooking, of seasoning, or of flavouring, or to any combination of such processes.

2. **Cured meat** is the clean, sound product obtained by subjecting meat to a process of salting, by the employment of dry common salt or of brine, with or without the use of one or more of the following: sodium nitrite, sodium nitrate, potassium nitrate, sugar, a syrup, honey, spice.

3. **Dry salt meat** is the prepared meat which has been cured by the application of dry common salt, with or without the use of one or more of the following: sodium nitrite, sodium nitrate, potassium nitrate, sugar, a syrup, honey, spice; with or without the injection into it of a solution of common salt to which may have been added one or more of the following: sodium nitrite, sodium nitrate, potassium nitrate, sugar, a syrup, honey.

4. **Corned meat** is the prepared meat which has been cured by soaking in, with or without injecting into it, a solution of common salt, with or without one or more of the following, each in its proper proportion: sodium nitrite, sodium nitrate, potassium nitrate, sugar, a syrup, honey; and with or without the use of spice.

5. **Sweet pickled meat** is the prepared meat which has been cured by soaking in, with or without injecting into it, a solution of common

salt with sugar, a syrup, and/or honey, together with one or more of the following, each in its proper proportion: sodium nitrite, sodium nitrate, potassium nitrate, and with or without the use of spice.

6. **Dried meat** is the clean, sound product obtained by subjecting fresh meat or cured meat to a process of drying, with or without the aid of artificial heat, until a substantial portion of the water has been removed.

7. **Smoked meat** is the clean, sound product obtained by subjecting fresh meat, dried meat, or cured meat, to the direct action of the smoke either of burning wood or of similar burning material.

8. **Canned meat** is fresh meat or prepared meat, packed in hermetically sealed containers, with or without subsequent heating for the purpose of sterilisation.

9. **Hamburg steak, "hamburger steak,"** is comminuted fresh beef, with or without the addition of suet and/or of seasoning.

10. **Potted meat, deviled meat,** is the clean, sound product obtained by comminuting and cooking fresh meat and/or prepared meat, with or without spice, and is usually packed in hermetically sealed containers.

11. **Sausage meat** is fresh meat or prepared meat, or a mixture of fresh meat and prepared meat, and is sometimes comminuted. The term "sausage meat" is sometimes applied to bulk sausage containing no meat-by-products.

#### (d) MEAT FOOD PRODUCTS

1. **Meat food products** are any articles of food or any articles that enter into the composition of food, which are not prepared meats, but which are derived or prepared, in whole or in part, by a process of manufacture from any portion of the carcasses of cattle, swine, sheep, or goats, if such manufactured portion be all, or a considerable and definite portion, of the article—except such preparations as are for medicinal purposes only.

2. **Meat loaf** is the product consisting of a mixture of comminuted meat with spice and/or with cereals, with or without milk and/or eggs, pressed into the form of a loaf and cooked.

3. **Pork sausage** is chopped or ground pork, with or without one or more of the following: herbs, spice, common salt, sodium nitrite, sodium nitrate, potassium nitrate, sugar, a syrup, water, vinegar; and may be fresh, dried, smoked, or cooked.

The definition of other types of sausage was postponed for further consideration.

4. **Brawn** is the product made from chopped, or ground, and cooked edible parts of swine, chiefly from the head, feet, and/or legs, with or without the chopped or ground tongue.

5. **Head cheese, mock brawn**, differs from brawn in that other meat and/or meat by-products are substituted, in whole or in part, for corresponding parts derived from swine.

6. **Souse** is the product consisting of meat and/or meat by-products; after cooking, the mixture is commonly packed into containers and covered with vinegar.

7. **Scrapple** is the product consisting of meat and/or meat by-products mixed with meal or the flour of grain, and cooked with seasoning materials, after which it is poured into a mold.

These definitions were recommended by the Food Standards Committee, a joint committee including representatives of the Association of American Dairy, Food and Drug Officials, Association of Official Agricultural Chemists and the United States Department of Agriculture.

The standards and definitions recommended by this committee are not only adopted for the guidance of its officials in enforcing the Federal Food and Drugs Act, but by many States in the enforcement of State laws. In some States the standards and definitions adopted by the Department of Agriculture automatically become standards and definitions for those States.

While primarily intended for the guidance of officials of the United States Department of Agriculture in the enforcement of the Federal Food and Drugs Act and in the carrying out of the duties of the Bureau of Animal Industry, these definitions may be found useful in other English-speaking countries.

The definition for fresh meat would include not only meat chilled sufficiently to produce a thoroughly merchantable commercial article, but also that stored at temperatures just above the freezing point of meat, providing such storage produces no substantial change in character. It will also include meat that has been frozen and held in the frozen state, provided the process employed produces no substantial change in character and provided the meat reaches the consumer in good condition. The various questions arising in this

connection will be discussed later in connection with the effects of chilling, freezing, and storage.

The principal large animals from which civilised countries derive their meat supply are oxen, swine, sheep, goats, and horses. The first three are by far the most important. In Alaska, Canada, and the United States reindeer are used to some extent and are considered a domestic animal, Alaska and parts of Canada being the source of such meat. Wild game is consumed in relatively small amounts. Among the other animal foods, those from the domestic fowl and eggs take first rank, while fish and other sea foods complete the list.

Goats are used for food purposes to only a slight extent, and horses are slaughtered for food in comparatively few localities, chiefly in Europe. In the United States of America and in England horse meat is used only for feeding domestic animals and animals in various zoological gardens. On the other hand, the use of meat derived from oxen, swine, and sheep is practically universal, excepting where forbidden by religious injunction.

### Cuts of Meat

The methods of cutting up the carcasses of animals vary considerably in different localities and countries and are determined by convenience, custom, and subsequent handling. Naturally, they are different for different kinds of animals and for different ages and weights of the same kind of animal. The cuts into which the ordinary food animals are commonly divided are shown in the accompanying illustration. (Figure 9.) The carcasses are divided in the so-called Chicago style, which is being rather widely used in the United States of America. The cuts of beef are shown on a larger scale in Figures 10 and 11.

For more extended treatment of market classes and grades of livestock and meat the reader is referred to the various nutrition bulletins of the Office of Experiment Stations, United States Department of Agriculture; to Farmer's Bulletin No. 34; to Illinois Agricultural Experiment Station, Bulletin 158, 1912 (excellent illustrations and tables); to United States Department of Agriculture, Circular 28, 1928; and to the following Department Bulletins of the United States Department of Agriculture: No. 1246, 1924; No. 1360, 1927; No. 1464, 1927; and No. 1470, 1927.



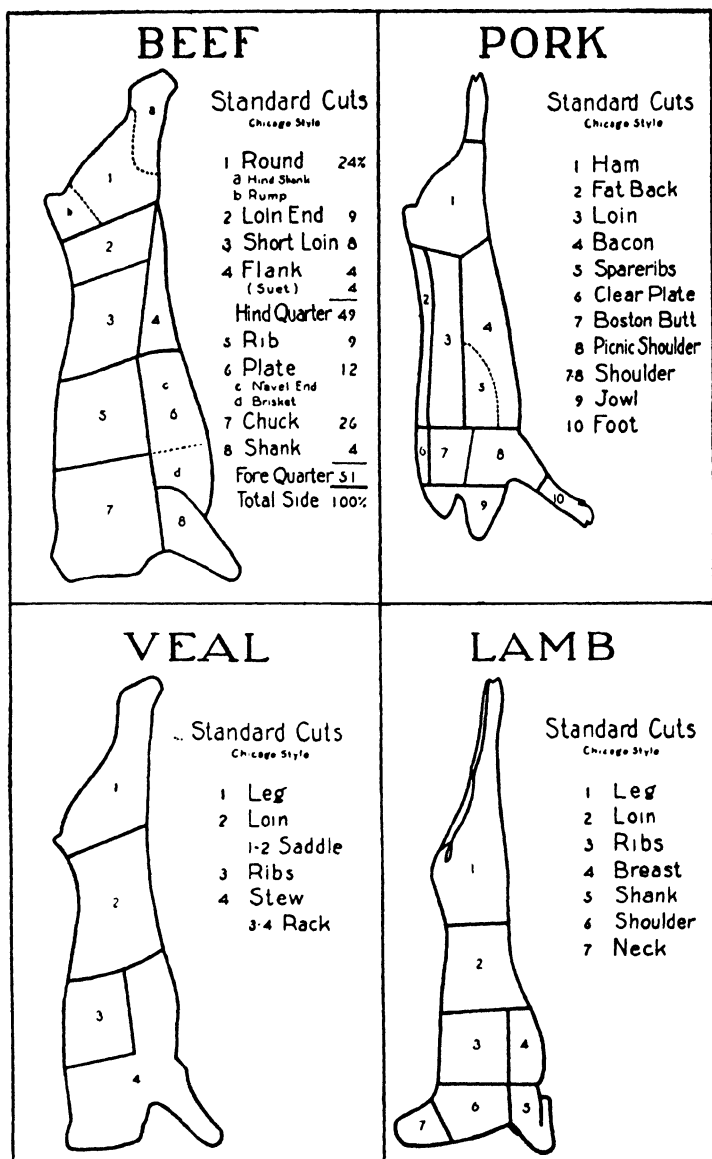


FIG. 9.—Cuts of meat.

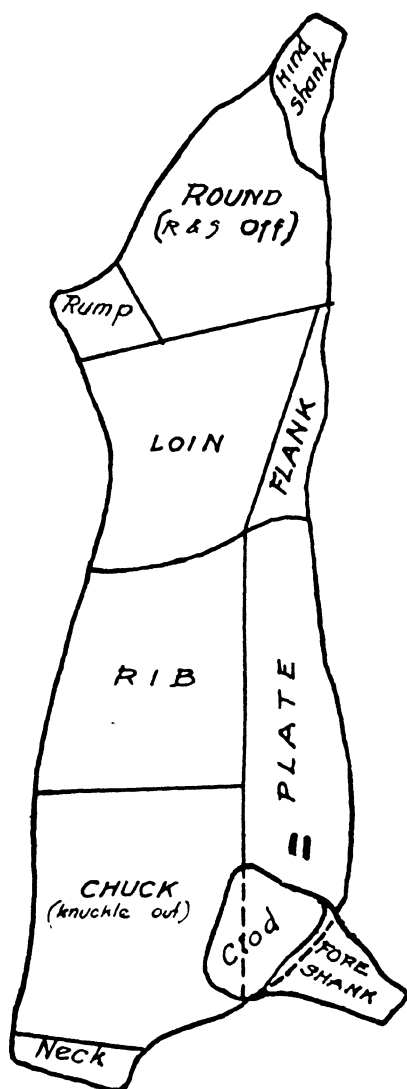


FIG. 10.—Cuts of beef. (Bull. 158, Illinois Exp. Sta.)

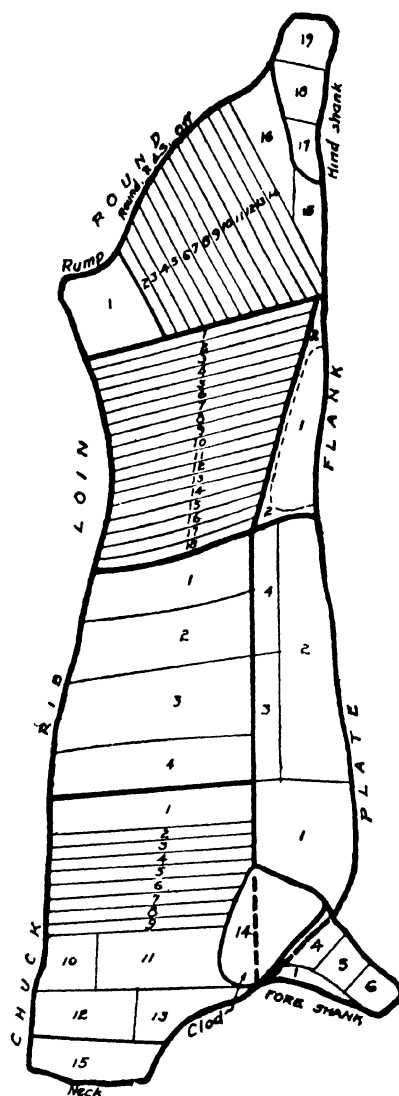


FIG. 11.—Retail cuts of beef. (Bull. 158, Illinois Exp. Sta.)

### Composition of Meat

It is customary in meat-packing houses, in agricultural experiment stations, and in other places interested in securing information on yields of meat by meat-producing animals, to secure data under the following headings: live weight, dressed weight, and weights of blood, viscera, offal, lean meat, fatty tissue, and bone. In some cases it is important in getting the relations of certain parts to the whole to secure also the warm empty weight, or the live weight minus the food, fæces, and urine.

In determining the chemical composition of the parts it is customary to determine the moisture, fat, protein, and ash according to methods to be discussed later. Sometimes, especially in research work, it is desirable to know more about the proteins, mineral constituents, and even the vitamins occurring in the meat or meat product. The most common determinations made in this latter connection have to do with the distribution of the proteins or other nitrogen-containing substances. This is especially true in connection with the determination of changes due to storage or to spoilage and in the case of meat extracts and similar preparations.

### WORK OF EARLIER INVESTIGATORS

The gross composition of the principal food animals has been treated at length by Lawes and Gilbert (*Phil. Trans.*, 1859, 2, 494), by Wiley (*Div. Chem. U. S. Dept. Agr. Bull.* 53), and by Atwater and Bryant (*U. S. Dept. Agr. Off. Exp. Sta. Bull.* 28, Revised).

The following is the percentage composition of the lean of some of the principal kinds of flesh used for food (Munk's *Physiologie*):

	Ox	Calf	Pig	Horse	Fowl
Water.....	76.7	75.6	72.6	74.3	70.8
Proteins and gelatin.....	20.0	19.4	19.9	21.6	22.7
Fat.....	1.5	2.0	6.2	2.5	4.1
Carbohydrates.....	0.6	0.8	0.6	0.6	1.3
Salts.....	1.2	1.3	1.1	1.0	1.1

Hence, the lean of meat contains 6 times the proportion of proteins present in milk, and about the same proportion as is contained in the white of egg (page 542).

The following analyses, showing the average composition of fresh meat, are by König:

Description of meat	No. of samples contributing to average	Water	Nitrogenous matters	Fat	Ash
Very fat ox-flesh.....	7	55.42	17.19	26.38	1.08
Moderately fat ox-flesh.....	21	73.25	20.78	5.33	1.33
Lean ox-flesh.....	9	76.71	20.78	1.50	1.18
Fat cow-flesh.....	9	70.98	19.86	7.70	1.07
Lean cow-flesh.....	6	76.35	20.54	1.78	1.32
Very fat mutton.....	3	47.91	14.80	36.39	0.85
Moderately fat mutton.....	8	75.99	17.11	5.77	1.33
Horse-flesh.....	12	74.27	21.71	2.55	1.01

A. H. Church (*Food; Some Account of Its Sources, Constituents and Uses*) gives the following as the composition of a *mutton-chop*, exclusive of the bone, when quite fresh: Water, 44.1; albumin, 1.7; fibrin (true muscle), 5.9; ossein-like substances, 1.2; fat, 42.0; organic extractives, 1.8; mineral matters, 1.0; and other substances, 2.3%.

The following analyses of *animal foods* are also by Church:

	Water	Nitrogenous matters	Fat	Mineral matter	Remarks
Tripe.....	79.5	10.0	10.0	0.5	The sample was cleansed, boiled, and freed from excess of fat.
Fowl.....					The nitrogenous matters included ossein-like substances.
Streaky bacon.....	22.3	8.1	65.2	4.4	The ash includes 3.8% of common salt.
Mackerel.....	68.7	13.5	12.5	3.3	The ash includes 0.2% of common salt.

J. König gives the following percentage analyses of the flesh of *wild animals* and of *birds*:

	Water	Nitrogenous matters	Fat	Other nitrogen-free substances	Ash
Hare.....	74.16	23.34	1.13	0.19	1.18
Rabbit.....	66.85	21.47	9.76	0.75	1.17
Deer.....	75.76	19.77	1.92	1.42	1.13
Domestic hen.....	76.22	19.72	1.42	1.27	1.37
Wild duck.....	70.82	22.65	3.11	2.33	1.09
Partridge.....	71.96	25.26	1.43	.....	1.39
Pigeon.....	75.10	22.14	1.00	0.76	1.00

The following analyses of *animal foods* are due to Payen:

Food	Water, per cent	Nitrogenous matters, per cent	Carbohydrates, etc., per cent	Fat, per cent	Ash, per cent
Calves' liver.....	72.33	20.10	0.45	5.58	1.54
Sheep's kidneys.....	78.20	17.25	1.32	2.12	1.10
Foie gras.....	22.70	13.75	6.40	54.57	2.58
Lobster (fresh).....	76.62	19.17	1.22	1.17	1.17
Oysters.....	80.38	14.01	1.40	1.51	2.69
Mussels.....	75.74	11.72	7.39	2.42	2.73

Lawes and Gilbert, in their elaborate essay, "On the Composition of Some of the Animals Fed and Slaughtered as Human Food" (*Phil. Trans.*, 1859, 2, pages 493 to 680) give a large number of analyses showing the composition of the entire animals and of various parts thereof. Their results show that the total edible parts of the ten animals analysed contained 3.5 parts of fat for 1 of dry nitrogenous matter.

#### PERCENTAGE COMPOSITION OF FLESH—LAWES AND GILBERT

	Medium ox	Half fat ox	Fat ox	Fat calf	Thin sheep	Medium sheep	Half fat sheep	Fat sheep	Very fat sheep	Medium hog	Fat hog
Water.....	73.7	64.4	55.3	70.6	75.0	72.8	65.2	54.0	46.0	60.9	44.4
Fat.....	5.3	17.2	29.4	11.3	5.7	0.0	19.5	33.6	43.2	20.2	45.5
Proteins.....	19.8	17.5	14.5	17.0	18.0	17.1	14.5	11.7	10.2	12.3	9.7
Ash.....	1.2	0.9	0.8	1.1	1.3	1.1	0.8	0.7	0.6	0.6	0.4

A. H. Church cites the following figures illustrating the composition of cooked *mutton chops*. The 2 analyses are evidently quite independent, and do *not* represent the composition of the same chop, with and without the gravy and dripping.

	Water	Nitrogenous matters	Fat	Mineral matter	Other substances
Cooked chop, including gravy and dripping, per cent. ....	54 0	27.6	15.4	3.0 <sup>1</sup>	.....
Cooked chop, exclusive of gravy and dripping, per cent. ....	51.6	36.6	9.4	1.2	1.2

<sup>1</sup> This figure is evidently an estimation by difference, and is in excess of the truth, unless about 2 per cent salt had been added in the cooking.

The following results were obtained in Allen's laboratory by A. R. Tankard. They represent the composition of various kinds of meat cut from the cold roast joint, and wholly edible. They include such a proportion of fat as would be commonly served and eaten with the lean, but are exclusive of skin, gravy, and dripping.

	Mutton %	Lamb %	Beef %	Veal %	Pork %	Duck %	Fowl %
Water (loss at 100°).....	39.76	59.89	45.63	51.88	44.90	64.13	67.40
Fat (ether extract).....	26.80	11.95	24.21	11.39	19.67	6.06	6.68
Proteins (N × 6.3).....	29.04	24.69	26.50	32.19	32.63	27.12	24.26
Ash (sulphated).....	1.93	1.63	1.21	1.57	1.86	2.04	1.37
	97.53	98.16	97.55	97.03	99.06	99.35	99.71
Cold water extract.....	3.74	2.81	3.60	6.55	.....	3.70	4.00
Containing ash.....	0.97	0.92	1.10	1.30	.....	1.20	0.60

Grindley and his coworkers have published many tables of analyses showing the composition of cooked meats (see page 314).

Henneberg, Kern, and Wattenberg (*J. f. Landw.*, 1878, 26, 549; 1880, 28, 289; *Z. f. Biol.*, 1881, 17, 295) have reported the composition of the entire flesh of seven wholesale cuts of the carcasses of eight sheep. The cuts do not correspond to those in use now. The nitrogen was determined by the Will-Varrentrapp method, which employs soda-lime and oxalic acid. For these and other reasons, it does not seem worth while presenting their detailed results. The following summary will suffice.

## PERCENTAGE COMPOSITION OF THE ENTIRE FLESH OF THE CARCASSES OF SHEEP

(Henneberg, Kern, and Wattenberg)

Age in months	Condition	Empty wt., kilo-grams	Water %	Fat %	Protein %	Ash %
6½	Thin.....	14.91	68.45	13.08	17.19	1.06
12½	Fat.....	39.50	66.62	15.90	17.06	0.99
13	Plump.....	31.97	68.98	13.16	17.56	1.00
17½	Very fat.....	52.94	57.63	25.80	15.88	0.95
21½	Plump and fat.....	48.97	62.16	20.58	16.63	0.97
27½	Very fat.....	61.66	63.49	18.41	17.25	1.03
33	Almost plump.....	35.75	59.93	23.71	15.56	1.00
40	Very fat.....	51.90	66.29	14.98	18.06	1.04

The following tables were compiled by A. E. Leach (*Food Inspection and Analysis*, pages 213-16) from Atwater and Bryant's figures

## PERCENTAGE COMPOSITION OF BEEF

Cut	Number of analyses	Refuse	Water	Protein		Fat	Ash	Fuel value per pound cals.
				N X 6.25	By difference			
Chuck: Lean—Edible portion.....	2	.....	71.3	20.2	19.5	8.2	1.0	720
As purchased.....	2	19.5	57.4	16.3	15.7	6.6	0.8	570
Medium—Edible portion.....	4	.....	68.3	19.6	18.9	11.9	0.9	865
As purchased.....	4	15.2	57.9	16.6	16.0	10.1	0.8	735
Fat—Edible portion.....	4	.....	62.3	18.5	18.0	18.8	0.9	1135
As purchased.....	3	14.7	53.1	15.9	15.4	15.9	0.7	965
Ribs: Lean—Edible portion.....	6	.....	66.0	16.5	16.9	9.8	0.8	790
As purchased.....	6	22.6	52.6	15.2	14.8	9.3	0.7	675
Medium—Edible portion.....	15	.....	55.5	17.5	17.0	26.6	0.9	1450
As purchased.....	15	26.8	43.8	13.9	13.5	21.2	0.7	1155
Fat—Edible portion.....	9	.....	48.5	15.0	15.2	35.6	0.7	1780
As purchased.....	8	16.8	39.6	12.7	12.4	36.6	0.6	1525
Loin: Lean—Edible portion.....	12	.....	67.0	19.7	19.3	12.7	1.0	900
As purchased.....	11	13.1	58.2	17.1	16.7	11.1	0.9	785
Medium—Edible portion.....	32	.....	60.6	18.5	18.2	20.2	1.0	1190
As purchased.....	32	13.3	52.5	16.1	15.8	17.5	0.9	1040
Fat—Edible portion.....	6	.....	54.7	17.5	16.8	27.6	0.9	1490
As purchased.....	6	10.2	49.2	15.7	15.0	24.8	0.8	1305
Rump: Lean—Edible portion.....	4	.....	65.7	20.9	19.6	13.7	1.0	965
As purchased.....	3	14.0	50.6	19.1	17.5	11.0	0.9	820
Medium—Edible portion.....	10	.....	56.7	17.4	16.9	25.5	0.9	1400
As purchased.....	10	20.7	45.0	13.8	13.4	20.2	0.7	1110
Fat—Edible portion.....	5	.....	47.1	16.8	16.4	35.7	0.8	1820
As purchased.....	5	23.0	30.2	12.9	12.6	27.6	0.6	1405
Round: Lean—Edible portion.....	31	.....	70.0	21.3	21.0	7.9	1.1	730
As purchased.....	29	8.1	64.4	19.5	19.2	7.3	1.0	670
Medium—Edible portion.....	18	.....	65.5	20.3	19.8	13.6	1.1	950
As purchased.....	14	7.2	60.7	17.0	16.3	12.8	1.0	895
Fat—Edible portion.....	5	.....	60.4	19.5	19.1	19.5	1.0	1185
As purchased.....	3	12.0	54.0	17.5	17.1	16.1	0.8	1005



## PERCENTAGE COMPOSITION OF VEAL

Cut	Number of analyses	Refuse	Water	Protein		Fat	Ash	Fuel value per pound, cals.
				N X 6.25	By difference			
Chuck: Lean—Edible portion.....	1	.....	76.3	.....	20.6	1.9	1.2	465
As purchased.....	1	19.0	61.8	.....	16.7	1.6	0.9	380
Medium—Edible portion.....	6	.....	73.3	19.7	19.2	0.5	1.0	640
As purchased.....	6	18.9	59.5	16.0	15.6	5.2	0.8	515
Ribs: Medium—Edible portion.....	9	.....	72.7	20.7	20.1	6.1	1.1	640
As purchased.....	9	25.3	54.3	15.5	15.0	4.6	0.8	480
Fat—Edible portion.....	3	.....	60.9	18.7	18.8	19.3	1.0	1160
As purchased.....	3	24.3	46.2	14.2	14.2	14.5	0.8	875
Loin: Lean—Edible portion.....	5	.....	73.3	20.4	19.9	5.6	1.2	615
As purchased.....	5	22.0	57.1	15.9	15.6	4.4	0.9	480
Medium—Edible portion.....	6	.....	69.0	19.9	19.2	10.8	1.0	825
As purchased.....	6	16.5	57.6	16.6	16.0	9.0	0.9	690
Fat—Edible portion.....	2	.....	61.6	18.7	18.5	18.9	1.0	1145
As purchased.....	2	18.3	50.4	15.3	15.1	15.4	0.8	935
Leg: Lean—Edible portion.....	9	.....	73.5	21.3	21.2	4.1	1.2	570
As purchased.....	9	9.1	66.8	19.4	19.3	3.7	1.1	520
Medium—Edible portion.....	10	.....	70.0	20.2	19.8	9.0	1.2	755
As purchased.....	9	14.2	60.1	15.5	16.9	7.9	0.9	620

## PERCENTAGE COMPOSITION OF MUTTON AND LAMB

Cut	Number of analyses	Refuse	Water	Protein		Fat	Ash	Fuel value per pound cals.
				N X 6.25	By difference			
<i>Mutton.</i>								
Chuck: Lean—Edible portion.	1	.....	64.7	17.8	18.1	16.3	0.9	1020
As purchased.	1	19.5	52.1	14.3	14.5	13.1	0.8	820
Medium—Edible portion.	6	.....	50.9	15.1	11.6	31.6	0.9	1700
As purchased.	6	21.3	39.9	11.9	11.5	26.7	0.6	1350
Fat—Edible portion.	2	.....	40.6	13.9	13.7	44.9	0.8	2155
As purchased.	2	16.5	31.8	11.6	11.5	37.5	0.7	1800
Loin: Medium—Edible portion.	13	.....	50.2	16.0	15.9	31.1	0.8	1695
As purchased.	12	16.0	42.0	13.5	11.0	28.3	0.7	1445
Fat—Edible portion.	3	.....	43.3	14.7	11.2	41.7	0.8	2035
As purchased.	3	11.7	38.3	13.0	12.5	36.8	0.7	1705
Flank: Medium—Edible portion.	8	.....	46.2	15.2	14.8	38.3	0.7	1900
As purchased.	2	9.9	39.0	11.8	11.6	36.9	0.6	1815
Leg: Lean—Edible portion.	3	.....	67.4	19.8	19.1	12.4	1.1	890
As purchased.	3	16.8	56.1	16.5	15.9	10.3	0.9	740
Medium—Edible portion.	11	.....	62.8	18.5	18.2	18.0	1.0	1105
As purchased.	11	18.4	51.2	15.1	14.9	14.7	0.8	900
<i>Lamb.</i>								
Chuck: Edible portion.	1	.....	56.2	19.1	19.2	23.6	1.0	1350
As purchased.	1	19.1	45.5	15.4	15.5	19.1	0.8	1090
Leg: Medium—Edible portion.	2	.....	63.9	19.2	18.5	16.5	1.1	1055
As purchased.	2	17.4	52.9	15.9	15.2	13.6	0.9	870
Fat—Edible portion.	1	.....	54.6	18.3	17.1	27.4	0.9	1405
As purchased.	1	13.4	47.3	15.8	14.8	23.7	0.8	1295
Loin: Edible portion.	4	.....	53.1	18.7	17.6	28.3	1.0	1540
As purchased.	4	14.8	45.3	16.0	15.0	24.1	0.8	1315

## PERCENTAGE COMPOSITION OF PORK, POULTRY AND GAME

Cut	Number of analyses	Refuse	Water	Protein		Fat	Ash	Fuel value per pound cals.
				N X 6.25	By difference			
<i>Pork.</i>								
Shoulder: Edible portion.....	19	.....	51.2	13.3	13.8	34.2	0.8	1690
As purchased.....	19	12.4	44.9	12.0	12.2	29.8	0.7	1480
Loin: Lean—Edible portion.....	1	.....	60.3	20.3	19.7	19.0	1.0	1180
As purchased.....	1	23.5	46.1	15.5	15.1	14.5	0.8	900
Fat—Edible portion.....	4	.....	41.8	14.5	13.1	44.4	0.7	2145
As purchased.....	4	16.5	34.8	11.9	10.9	37.2	0.6	1790
Ham: Lean—Edible portion.....	2	.....	60.0	25.0	24.3	14.4	1.3	1075
As purchased.....	2	0.9	59.4	24.8	24.2	14.2	1.3	1060
Fat—Edible portion.....	5	.....	38.7	12.4	10.6	50.0	0.1	2345
As purchased.....	5	13.2	33.6	10.7	9.2	43.5	0.5	2035
<i>Poultry and Game.</i>								
Chicken: Edible portion.....	3	.....	74.8	21.5	21.6	2.5	1.1	505
As purchased.....	3	41.6	43.7	12.8	12.6	1.4	0.7	295
Powl: Edible portion.....	26	.....	63.7	19.3	19.0	16.3	1.0	1045
As purchased.....	26	25.9	47.1	11.7	14.0	12.3	0.7	775
Goose: Edible portion.....	1	.....	46.7	16.3	16.3	36.2	0.8	1830
As purchased.....	1	17.6	38.5	13.4	13.4	29.8	0.7	1505
Turkey: Edible portion.....	3	.....	55.5	21.1	20.6	22.9	1.0	1360
As purchased.....	3	22.7	42.4	16.1	15.7	18.4	0.8	1075
Quail: As purchased.....	1	.....	66.9	21.8	.....	8.0	1.7	775

## WORK OF RECENT INVESTIGATORS

The preceding results are those secured by investigators working largely in the nineteenth century. Considerable new work has been reported since that time. In many cases improved methods of analysis have been used, especially in the determination of total nitrogen, from which protein is calculated by the conventional factor, 6.25. A separate report of these results would seem to be justified.

**Veal.**—Washburn and Jones (*Vermont Agr. Expt. Sta. Bull.* 195, 1916) have reported the following composition of milk-fed veal.

## PERCENTAGE COMPOSITION OF FLESH OF MILK-FED VEAL

Age in days	Condition	Water	Fat	Protein
42	Poor	75.22	1.24	21.36
44	Poor	76.14	1.39	21.00
40	Fair	72.94	4.14	20.86
41	Good	74.75	4.86	19.75
35	Good	72.58	6.03	19.36
38	Good	73.86	5.82	19.37

**Beef.**—Hall and Emmett (*Ill. Agr. Expt. Sta. Bull.* 158, 1912) report the composition of the flesh of the wholesale cuts of a prime steer as follows:

PERCENTAGE COMPOSITION OF FLESH OF WHOLESALE CUTS OF A PRIME STEER

	Water	Fat	Protein	Organic extractives		Ash	Cals. per 100 gr.
				Nitrogenous	Non-nitrogenous		
Flank.....	32.26	57.16	9.44	.34	.42	.40	554.9
Plate.....	39.42	48.57	10.59	.46	.62	.51	483.1
Rib.....	45.15	40.62	12.32	.60	.75	.62	419.7
Rump.....	46.25	38.95	12.56	.63	.91	.64	405.8
Loin.....	47.42	37.71	12.96	.69	.88	.64	396.8
Chuck.....	55.47	27.54	14.87	.78	.90	.78	313.7
Neck.....	56.32	26.12	15.59	.70	.84	.75	303.0
Fore shank.....	60.95	19.98	16.98	.74	.99	.83	253.7
Hind shank.....	61.02	20.77	16.26	.75	.92	.81	257.9
Round.....	60.86	19.65	16.50	.94	1.11	.87	250.5
Clod.....	63.04	17.96	16.69	.94	1.12	.85	235.1

The most extensive work on beef cattle is that done at the University of Missouri by Moulton, Trowbridge, and Haigh (*Missouri Agr. Expt. Sta. Research Bulls.* 28, 1918; 30, 1919; 38, 1920; 55, 1922; 59, 1923; and 61, 1923). This work is too extensive to quote in detail. The following are some selected results for the entire flesh of the carcass of beef animals from birth to four years old. Three groups of cattle were analysed. The cattle of Group I were full fed at all times. Group II was fed for maximum growth without allowing for any appreciable fattening. Group III was fed so as to permit fairly normal growth in skeletal measurements while keeping the animal rather thin. These investigators have also reported the analyses of the lean, fatty tissue, and bone of the various wholesale cuts of beef, as well as the composition of the different viscera and offal.

**Pork.**—Numerous investigators have reported the composition of various samples of pork. Forbes (*Missouri Agr. Expt. Sta. Bull.* 81, 1909) gives the following composition for pork tenderloin. He also reports the composition of the kidneys, livers, and a cross

**PERCENTAGE COMPOSITION OF ENTIRE FLESH OF BEEF  
ANIMALS—MISSOURI WORK**

Group	Age in months	Water	Fat	Protein	Ash	Phosphorus
	<b>Calf at birth</b>	77.03	4.01	16.94	0.99	0.17
I	3	70.59	9.14	18.75	1.13	0.18
II	3	73.19	5.94	19.50	1.17	0.19
III	3	76.42	3.26	19.25	1.10	0.19
I	5½	58.12	24.82	15.50	0.86	0.16
II	5½	77.03	9.45	18.69	0.99	0.18
III	5½	73.75	4.73	20.00	1.05	0.19
I	8½	63.12	17.92	17.00	0.89	0.16
II	8½	66.53	14.30	17.69	0.94	0.19
III	8½	73.49	5.52	19.06	0.96	0.19
I	11	58.71	23.09	16.75	0.84	0.16
II	11	67.66	14.01	18.13	0.94	0.18
III	11	67.88	11.72	17.56	0.97	0.18
I	11	53.60	20.68	16.06	0.73	0.15
II	11	63.17	18.39	17.94	0.84	0.16
I	18	53.95	28.72	16.06	0.79	0.14
III	18	68.05	10.03	19.81	1.03	0.19
I	21	49.60	35.13	14.31	0.68	0.13
II	26	65.17	16.50	17.69	0.86	0.16
III	26	68.45	11.91	17.56	0.94	0.17
I	36	42.28	45.22	11.81	0.57	0.10
II	36	60.40	21.48	17.00	0.81	0.15
I	40	37.34	51.80	10.19	0.49	0.09
II	40	59.20	22.38	17.25	0.83	0.15
III	40	70.33	8.86	19.69	0.98	0.18
I	45	38.66	49.67	10.63	0.51	0.09
II	45	61.63	18.22	18.19	0.84	0.16
III	45	63.17	16.96	18.06	0.85	0.16
I	48	30.25	53.12	9.13	0.49	0.09
II	48	54.96	28.29	15.50	0.77	0.13
III	48	63.11	17.23	18.50	0.89	0.16
	<b>Cattle over 5 months fat free</b>	76.5	.....	21.88	1.1	0.20-22

**PERCENTAGE COMPOSITION OF PORK TENDERLOIN—FORBES**

Size of pig	Number of samples	Water	Fat	Protein	Ash
120 lbs. ....	65	73.24	5.03	20.28	1.12
120 lbs. ....	30	73.19	4.80	19.77	1.12
Younger pigs. ....	25	73.90	4.42	19.53	1.10

section of the carcass at the sixth rib. Later with Beigle, Fritz, Morgan, and Rhue (*Ohio Agr. Expt. Sta. Bull.* 283, 1915) he reports the composite of flesh of thirty-five pigs weighing from 100 to 225

pounds. The average composition of the flesh of all of the pigs is: water, 35.53; fat, 55.12; protein, 9.64; and ash, 0.58.

Emmet, Grindley, Joseph, and Williams in a series of publications (*Illinois Agr. Expt. Sta. Bulls.* 168, 169, 171, and 173, 1914) give the composition of the boneless meat from the ham, shoulder, and side of five pigs weighing 200 to 250 pounds. The average composition of the cuts of the five pigs is given in the accompanying table.

PERCENTAGE COMPOSITION OF BONELESS MEAT OF PORK  
CUTS—ILLINOIS WORK

Cut	Number of samples	Water	Fat	Protein	Ash	Phosphorus
Ham	5	51.55	32.37	15.29	0.79	0.15
Shoulder	5	48.05	37.46	13.73	0.76	0.13
Side	5	33.47	55.09	10.84	0.59	0.11

Wellman (*Landwirtsch. Jahrb.*, 1914, 46, 499) gives the composition of the lean flesh of eight pigs and of the entire bodies of five. For

PERCENTAGE COMPOSITION OF FLESH OF PIGS—MISSOURI DATA

Animal No.	Live wt. kg.	Water	Fat	Protein	Ash	Phosphorus	
Lean Flesh of Carcass							
1	105.8	59.70	20.95	17.81	0.83	0.16	End of gestation
2	113.4	65.07	15.31	20.00	1.00	0.17	69 days, lactation
3	111.8	64.00	16.88	17.56	0.88	0.14	Farrowed
4	74.2	69.37	13.70	18.25	0.96	0.16	Not bred
5	85.3	70.26	10.46	18.94	0.98	0.17	69 days, lactation
6	120.7	65.44	14.82	19.50	0.91	0.16	Not bred
7	112.5	64.14	15.35	18.94	0.97	0.17	88 days pregnant
8	110.5	64.04	17.17	19.06	0.90	0.17	Farrowed
9	128.8	60.11	20.37	18.69	0.93	0.15	69 days, no lactation
10	101.6	63.15	18.03	18.50	0.96	0.18	Not bred
Fatty Tissue of Carcass							
1	..	9.62	87.45	3.63	0.14	0.02	..
2	..	9.21	87.32	3.50	0.17	0.02	..
3	..	11.91	84.72	3.25	0.20	0.04	..
4	..	13.93	81.63	4.06	0.20	0.03	..
5	..	14.70	79.79	4.81	0.21	0.03	..
6	..	10.88	85.93	3.13	0.18	0.02	..
7	..	12.77	84.64	3.06	0.18	0.03	..
8	..	10.34	87.90	2.44	0.16	0.02	..
9	..	8.15	87.85	2.44	0.15	0.02	..
10	..	10.82	88.71	2.75	0.19	0.05	..

one pig the composition of the lean flesh is similar to that of a pig at birth. There must be some discrepancy which the author does not explain. For the five pigs for which the most complete data are given the ages varied from twenty-six to ninety-six days and the weights from 4.3 to 18.1 kilos. The lean muscle tissue of these five pigs contained on the average 76.19% water, 5.97% fat, 16.85% protein, and 1.01% ash.

Griswold, Trowbridge, Hogan, and Haigh (*Missouri Agr. Expt. Sta. Research Bull.* 114, 1928) report the composition of the lean flesh and of the fatty tissue of the carcasses of ten gilts in various stages of pregnancy and lactation, the effect of which is shown chiefly in the amount of fat carried on the carcass.

Mitchell and Hamilton (*Illinois Agr. Expt. Sta. Bull.* 323, 1929) report the results of a study of the composition of three types of hogs, namely, chubby, intermediate, and rangy. The flesh showed no differences in composition due to type. In calculating the protein these investigators preferred the factor 6.0, suggested by Armsby, to the customary factor 6.25.

#### PERCENTAGE COMPOSITION OF FLESH OF PIGS—ILLINOIS DATA

Number of animals	Average weight kg.	Sample	Water	Fat	Protein N × 6.0	Ash	Small calories per gr.
15	30	{ Lean meat	68.60	13.79	15.93	0.95	2.251
		{ Fatty tissue	29.51	61.66	7.00	0.36	6.144
15	22	{ Total flesh	69.69	12.68	15.77	0.84	2.154
		{ Lean meat	58.35	22.36	16.62	0.88	3.070
45	102	{ Fatty tissue	15.18	79.89	3.72	0.15	7.689

**Lamb and Mutton.**—Wright (*Trans. and Proc. New Zealand Inst.*, 1914, 47, 569) has analysed a carcass of mutton and one of lamb. His results are given in the accompanying table.

#### PERCENTAGE COMPOSITION OF LAMB AND MUTTON—WRIGHT

	Lamb			Mutton			
	Leg	Loin	Fore quarter	Leg	Loin	Shoulder	Neck and breast
Water.....	57.92	40.12	44.42	49.08	46.64	55.94	43.35
Fat.....	24.08	48.38	40.54	22.60	37.48	27.66	42.56
Protein.....	17.14	10.55	14.27	18.48	14.06	15.59	13.20
Ash.....	0.89	0.49	0.64	0.87	0.67	0.76	0.55

Trowbridge (unpublished work of the Missouri Agr. Expt. Sta.) analysed the lean flesh and fatty tissue of the carcasses of a lamb, a mature ewe, and a mature wether. His results follow.

PERCENTAGE COMPOSITION OF THE FLESH OF SHEEP—  
TROWBRIDGE

	Sample	Water	Fat	Protein	Ash
Lamb.....	Lean flesh	64.43	14.75	18.50	0.98
	Fatty tissue	7.66	89.10	3.88	0.19
Mature ewe.....	Lean flesh	68.91	9.14	19.88	1.00
	Fatty tissue	7.69	89.98	2.06	0.15
Mature wether.....	Lean flesh	67.93	12.77	19.00	0.98
	Fatty tissue	9.99	87.22	2.00	0.22

**Horse Flesh.**—Mitchell and Hamilton (*Illinois Agr. Expt. Sta. Report 1926-27*, 119) report the composition of the entire flesh of three horses, a stallion and two mares. The protein was determined by multiplying the nitrogen content by the conventional factor 6.25.

PERCENTAGE COMPOSITION OF HORSE FLESH—MITCHELL AND  
HAMILTON

Animal	Age in years	Wt. in pounds	Water	Fat	Protein	Ash	Calories per gr.
Stallion.....	22	1,525	63.8	15.1	20.9	0.91	2,507
Mare.....	17½	1,700	61.6	18.7	16.7	1.01	2,758
Mare.....	5	1,605	66.4	13.7	16.2	0.97	2,300

**Rabbit Flesh.**—Wilson reports some work done at the National Institute of Poultry Husbandry in England on the composition of rabbit flesh (*Harper-Adams Utility Poultry Jour.*, 1929, 14, 564)

PERCENTAGE COMPOSITION OF RABBIT FLESH

	Adult rabbits		Young rabbits	
	Does	Bucks	Does	Bucks
Water.....	62.98	66.93	67.30	69.68
Fat.....	9.12	3.29	5.80	1.74
Protein.....	19.96	21.88	20.27	21.11
Carbohydrate.....	6.68	6.57	5.21	5.96
Ash.....	1.27	1.33	1.42	1.55

It is not certain what is meant here by "carbohydrate." It must include the non-nitrogenous extractives. Since the analyses add up to 100 in two cases and miss this figure by 0.01 and 0.04 in the other cases, it may be safe to assume that the carbohydrate includes all matter not found in the other four classes, and that it was determined by difference.

**Composition of Meat Rations.**—Gephart and Lusk (*Analysis and Costs of Ready-to-serve Foods*, Chicago, 1915) have made a valuable study of 242 ready-to-serve foods, as dispensed by a well-known chain of restaurants in New York City. The various food portions were analysed, their calorific values determined, and a summary given of the cost of 2,500 calories in the various kinds of food purchased.

The composition and preservation of foodstuffs has been discussed by Razous (*L'Industrie Chimique*, 1920, 298, 342, and 381). A general description is given of the chemical composition, methods of preservation, and effects of refrigeration. Meat and fish are dealt with.

**Mineral Constituents.** The composition of the ash of the flesh of various animals has been compiled by Armsby and Moulton (*The Animal as a Converter of Matter and Energy*, The Chemical Catalog Co., New York, 1925, 34). The accompanying table is taken from their work. The results of Ali and Wolff show a low sulphur content. This is due to the method of ashing, whereby much sulphur was lost. The other investigators determined sulphur by methods which avoided loss.

From the analysis of the flesh of a large number of animals, J. Katz (*Archiv. gesam. Physiol.*, 1896, 63, 1) finds the ash constituents to vary between the following limits, in parts per 1,000 of the fresh flesh: potassium, 2.4 to 2.6; sodium, 0.3 to 1.5; calcium, 0.02 to 0.39; magnesium, 0.18 to 0.37; iron, 0.04 to 0.25, and chlorine, 0.32 to 0.8. The phosphorus from phosphates ranged from 1.22 to 2.04; from lecithin, 0.13 to 0.48; and from nuclein, from 0.09 to 0.32 parts per 1,000. The accompanying table (p. 230) gives a compilation of his results.

Albu and Neuberg (*Mineral Stoffwechsel*, Julius Springer, Berlin, 1906) discuss in detail the metabolism of the various mineral salts in the animal body and give tables of composition.

Forbes and Keith (*Ohio Agr. Expt. Sta., Tech. Bull.* No. 5, 1914) have published a very extensive review of the literature of phos-



## PERCENTAGE COMPOSITION OF ASH OF FLESH—COMPILED BY ARMSBY AND MOULTON

	Pig <sup>1</sup>	Beef, Calf <sup>2</sup>	Swine <sup>3</sup>	Cat- tles <sup>3</sup>	Calf <sup>3</sup>	Chick- ens <sup>3</sup>	Deer <sup>3</sup>	Rab- bit <sup>3</sup>	Dog <sup>3</sup>	Cat <sup>3</sup>	Frog <sup>3</sup>	Eel <sup>3</sup>	Pike <sup>3</sup>	Shell fish <sup>4</sup>	Hu- man <sup>5</sup>	Flesh <sup>4</sup>
FeO <sub>3</sub> .....	3.65	0.38	0.64	2.87	0.87	0.78	1.07	0.53	0.48	0.98	0.71	0.97	0.45	0.62	1.64	0.70
CaO.....	2.06	1.08	0.87	0.23	1.30	0.88	0.96	1.79	0.71	0.89	1.93	5.53	4.03	2.29	0.81	2.82
MgO.....	27.20	16.81	3.58	3.28	3.51	3.04	3.47	3.30	2.86	3.52	3.44	2.97	3.72	3.27	2.73	3.21
K <sub>2</sub> O.....	12.53	14.21	23.32	36.20	31.85	33.02	29.14	33.34	27.69	33.81	32.70	29.14	36.27	30.02	29.94	41.27
Na <sub>2</sub> O.....	33.07	36.48	37.12	31.59	35.00	34.19	40.99	40.29	37.56	34.24	37.58	40.79	35.09	23.34	36.16	42.54
P <sub>2</sub> O <sub>5</sub> .....	16.34	1.97 <sup>5</sup>	15.59	15.15	15.72	17.20	15.18	13.70	16.68	16.14	14.33	13.59	15.79	16.60	16.16	1.56 <sup>5</sup>
Co <sub>2</sub> .....	5.41	6.53	3.79	4.59	4.65	3.54	2.92	3.56	5.92	4.17	3.55	3.49	2.31	17.96	5.44	3.85
SiO <sub>2</sub> .....	101.22	98.41	83.1	101.00	100.80	100.66	100.80	100.80	101.33	100.94	100.80	100.70	100.52	104.04	101.22	100.69
O = Cl.....	1.22	1.48	0.84	1.04	1.06	0.80	0.66	0.80	1.33	0.94	0.80	0.70	0.52	4.04	1.22	0.87
Total.....	100.00	96.93	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.82

<sup>1</sup> Forbes, et al., Ohio Agr. Expt. Stat. Bul. 283 (1915). (Average calculated from 7 pigs).<sup>2</sup> Ali, Mohammed Ahmed, Dissertation, A. M., Univ. of Mo., 1922 (Average of three animals).<sup>3</sup> Katz, Julius, *Arch. ges. Physiol.*, (1866), 63, 1.<sup>4</sup> Wolff, "Aschen Analysen" (1871), 158.<sup>5</sup> Calculated as SO<sub>3</sub>.

## MINERAL CONSTITUENTS OF FLESH, PER CENT—KATZ

Flesh of	Water	Potassium oxide	Sodium oxide	Ferric oxide	Calcium oxide	Magnes- ium oxide	Total	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )		Chlorine	Sulphur
								Soluble in water	Soluble in alcohol		
Pig.....	72.89	0.306	0.210	0.008	0.011	0.046	0.487	0.150	0.085	0.051	0.294
Ox.....	75.80	0.441	0.083	0.015	0.003	0.040	0.389	0.379	0.065	0.046	0.187
Calf.....	75.39	0.458	0.160	0.013	0.020	0.050	0.503	0.334	0.097	0.072	0.226
Deer.....	75.27	0.405	0.095	0.018	0.013	0.048	0.509	0.411	0.096	0.062	0.211
Rabbit.....	76.83	0.479	0.067	0.008	0.026	0.048	0.579	0.469	0.068	0.043	0.199
Dog.....	76.42	0.392	0.127	0.006	0.010	0.039	0.512	0.345	0.110	0.055	0.227
Cat.....	75.14	0.456	0.097	0.013	0.012	0.047	0.461	0.351	0.066	0.043	0.219
Hen.....	68.38	0.560	0.123	0.013	0.015	0.061	0.580	0.456	0.057	0.067	0.292
Frog.....	81.62	0.371	0.074	0.009	0.027	0.039	0.426	0.349	0.047	0.030	0.103
Shellfish.....	80.60	0.403	0.133	0.008	0.031	0.044	0.313	0.229	0.021	0.041	0.223
Eel.....	63.10	0.290	0.045	0.008	0.055	0.030	0.336	0.046	0.023	0.038	0.136
Pike.....	79.83	0.391	0.040	0.006	0.050	0.051	0.485	0.392	0.036	0.058	0.246

phorus compounds in animal metabolism. A very complete bibliography is given.

Hogan and Nierman (*Missouri Agr. Expt. Sta. Research Bull.* 107, 1927) have reported the results of the analyses of the ash obtained from samples of the total flesh of the carcasses of a number of the beef cattle analysed over a period of years at the Missouri Agricultural Experiment Station. The animals varied in age from three to forty-eight months and included both lean and fat animals. There seemed to be no differences due to age or fatness. The results shown are the average of the results from seventeen cattle. The

PERCENTAGE COMPOSITION OF THE ASH OF THE FLESH OF CATTLE—MISSOURI WORK

	Na <sub>2</sub> O	K <sub>2</sub> O	CaO	Fe <sub>2</sub> O <sub>3</sub>	MgO	P <sub>2</sub> O <sub>5</sub>	Cl	SO <sub>3</sub>	SiO <sub>2</sub>	
Ash of flesh.....	12.22	35.20	1.84	0.87	3.06	37.69	5.23	2.00	1.14	
	Na	K	Ca	Fe	Mg	P	Cl	S	Si	S <sup>1</sup>
Fresh meat....	0.080	0.261	0.012	0.0051	0.016	0.146	0.047	0.0075	0.052	0.121
Fat-free fresh meat	0.098	0.318	0.014	0.0065	0.020	0.178	0.057	0.0084	0.087	0.160

<sup>1</sup> Analyses of fresh samples, avoiding loss.

method of ashing resulted in losses of both chlorine and sulphur. No precautions were taken to avoid added iron from the utensils and grinders used. For this reason the iron content is too high.

**Iron in Meats.**—Most of the iron figures reported under the analysis of the ash of flesh are not satisfactory for purposes of determining the iron content of meats. While these tissues are low in calcium, they are sufficiently high in both phosphorus and iron to be considered excellent sources of these two essential elements. Sherman (*Chemistry of Food and Nutrition*, Macmillan and Co., New York, 1927, 344) presents some figures for the iron content of meats and shows that it may be estimated from the protein content, the iron content per 100 gm. of protein being 0.015 gm. By his method of calculation lean beef with a protein content of 20% would contain 0.003% of iron. Moulton (*American Food Journal*, December, 1923) has calculated from these data that a typical round steak would contain 0.0034% of iron, a typical porter-house steak would contain 0.0035%, a typical sirloin steak would contain 0.0030%, and a typical "chuck" roast would contain 0.0031% of iron.

Forbes and Swift have largely met the deficiency in our knowledge of the iron content of meats by analysing various meats prepared

and ground with knives and grinding machines free of iron. By observing other precautions they entirely avoided any outside contamination. Their results (*J. Biol. Chem.*, 1926, **67**, 517) are given in the accompanying table.

THE PERCENTAGE IRON CONTENT OF MEATS—FORBES AND SWIFT

	Water	Fat	Protein	Iron
Cured bacon.....	22.31	64.10	10.44	0.0013
Cured ham.....	64.61	13.60	21.06	0.0014
Pork shoulder.....	67.14	14.41	18.31	0.0015
Pork hindquarter.....	69.07	10.54	19.63	0.0015
Lamb shoulder.....	72.95	6.94	18.81	0.0016
Lamb hindquarter.....	75.59	5.27	20.31	0.0016
Beef rib.....	72.70	6.05	20.25	0.0024
Beef round.....	74.65	3.48	22.10	0.0025
Beef loin.....	72.02	6.39	20.50	0.0025
Beef chuck.....	72.84	7.13	19.81	0.0025
Veal forequarter.....	76.08	1.70	21.69	0.0023
Veal hindquarter.....	76.68	1.54	22.13	0.0027
Veal kidney.....	77.82	4.17	16.31	0.0040
Beef heart.....	78.91	4.94	16.19	0.0044
Beef brain.....	79.15	7.80	10.50	0.0053
Beef liver.....	68.33	4.66	20.63	0.0082
Beef spleen.....	77.50	1.90	18.04	0.0138
Beef kidney.....	78.67	1.85	16.13	0.0188
Beef blood.....	80.99	.....	17.94	0.0444

In determining the iron Forbes and Swift took portions of about 50 grm., weighed out into porcelain dishes at the time of grinding. These were covered with watch-glasses and put into a cold storage room, where they remained in a frozen condition until the determinations could be made. Samples for ether extract, moisture, and nitrogen determinations were also taken at the same time. All determinations represent at least three agreeing results. The samples for the iron determination were ignited to a white ash which was dissolved in hydrochloric acid, the iron being determined by titration with potassium permanganate solution of about 0.005 *N*. The triplicate results all agreed within 0.0001% of iron. In ashing meats trouble often results from spattering, especially when the samples are high in fat content. The samples were ashed without spattering by first drying in an electric oven and then ashing in an electric muffle furnace, the heat being carefully controlled with the aid of a thermo-electric pyrometer. Both porcelain and platinum dishes

were found unpractical, the former owing to the glaze which fused with the ash, and the latter owing to the phosphorus of the tissues attacking the platinum in the presence of much organic matter. Silica dishes were quite satisfactory.

Elvehjem and Hart (*J. Biol. Chem.*, 1926, **67**, 43) have made a study of methods for the determination of iron in biological materials. They recommend the following method.

*Standard Iron Solution.*—Dissolve 0.7 gram. of ferrous ammonium sulphate (dried to constant weight) in 100 c.c. of distilled water, and add 5 c.c. of concentrated sulphuric acid. Warm the solution slightly and add potassium permanganate until the iron is completely oxidised. Dilute the solution to 1 litre. One c.c. of the standard iron solution equals 0.1 mg. of Fe.

*A 10% solution of potassium thiocyanate.*

*N/5 Potassium Permanganate.*—Dissolve 6.30 gram. of the salt in distilled water and dilute to 1 litre.

*Hydrochloric Acid.*—Concentrated, free from iron.

*Nitric Acid.*—Concentrated, free from iron.

*Molybdate Solution.*—A solution of ammonium molybdate prepared in the usual manner, on which a blank iron determination has been made to insure its freedom from iron.

*Solution of Potassium Hydroxide.*—Prepared to be iron-free by making a 40% solution, allowing it to stand several days, and decanting the iron-free solution from the top.

*Procedure.*—A sample of the material to be analysed is weighed out so as to contain between 0.1 and 0.3 mg. of iron. Liquids are first evaporated to dryness. The sample is then carefully ignited in an electric furnace. A platinum dish is preferred by these investigators, although they add that a previously ignited and acid-washed porcelain evaporating dish may be used successfully. The ash is taken up in about 10 c.c. of water and 5 c.c. of concentrated hydrochloric acid and allowed to stand for several hours. The residue is filtered off and the phosphorus removed from the filtrate in the usual manner, which consists in adding concentrated ammonia until the solution becomes cloudy, clearing up with concentrated nitric acid, and adding 10 drops of nitric acid in excess. Then 30 c.c. of ammonium molybdate solution are added, the mixture heated on a water bath at 65° for half-an-hour, and the yellow precipitate filtered off. The precipitate is carefully washed with dilute nitric acid (9 c.c. of  $\text{HNO}_3$  in 100 c.c.

of water) to insure the removal of the last traces of iron from the precipitate to the filtrate. The solution is heated almost to boiling, and 40% potassium hydroxide solution (iron-free) is added until no further precipitate forms. Usually about 20 c.c. are required. The solution is boiled for several minutes to remove the ammonia present. The solution is then allowed to cool, and if the hydroxides do not settle properly, a few c.c. of potassium hydroxide solution are added and the liquid heated further. The precipitate is filtered off on an asbestos Gooch crucible, which has been carefully washed with hydrochloric acid, by decanting the clear liquid first and finally adding the precipitate to the Gooch crucible. The precipitate is washed with very dilute potassium hydroxide solution (1 to 2%). The best results are obtained if only a low pressure is maintained on the suction flask during filtering. The precipitate is dissolved from the Gooch crucible with 2.5 c.c. of concentrated hydrochloric acid, which is added in several portions (a few drops at a time), washing with water after each addition of acid. In this way the iron may be dissolved off completely and the total filtrate kept below 30 to 35 c.c. The best method of handling this small amount of solution is to introduce a test tube into the suction flask, allowing the end of the funnel to reach into the test tube so that the solution will be caught in the test tube instead of in the suction flask. The solution in the test tube is then washed into the original beaker, and the iron determined colorimetrically by adding enough  $N/5$  potassium permanganate solution to produce a faint pinkish colour (usually 1 to 2 drops) then adding 5 c.c. of a 10% solution of potassium thiocyanate, and making up to 50 c.c. in a volumetric flask. The colour produced is compared in a Duboscq colorimeter with a standard colour developed by taking 1 c.c. of the standard iron solution, adding 1 to 2 drops of  $N/5$  potassium permanganate solution and 5 c.c. of the 10% solution of potassium thiocyanate, and making up to 50 c.c. volume. The standard solution is set at 20 in the Duboscq colorimeter, and the unknown adjusted until the colours match. If the variation in the readings of the standard and unknown is too great, a smaller or larger amount of the standard should be taken, as the case may be.

The method given is based on that developed by Thomson (*J. Chem. Soc.*, 1885, 47, 493) but provides for the removal of phosphorus, which otherwise would interfere with the colour stability. Walker's method (*Analyst*, 1925, 50, 279), employing nitric acid as

the ash solvent, was found useful for such material as cabbage, but it could not be used where much phosphorus was present. A method of removing calcium, which sometimes makes the filtration of the hydroxides difficult, is given.

Some of the results obtained by the use of this method and reported by Elvehjem and Peterson (*J. Biol. Chem.*, 1927, **74**, 433) are given in the accompanying table.

# IRON CONTENT OF FRESH ANIMAL TISSUE—ELVEHJEM AND PETERSON

	Per cent
Beef	
Bone marrow.....	0.0009
Brain.....	0.0023
Heart.....	0.0048
Kidney.....	0.0055
Liver.....	0.0083
Lung.....	0.0122
Round steak muscle.....	0.0041
T-bone steak muscle.....	0.0037
Pancreas.....	0.0060
Spleen.....	0.0089
Rabbit muscle.....	0.0020

		Number of samples	Average %	Maximum %	Minimum %
Spleen.....	Beef	20	0.0091	0.0070	0.0118
	Calf	19	0.0255	0.0162	0.0384
	Hog	1	0.0204		
Liver.....	Beef	4	0.0083	0.0081	0.0085
	Calf	4	0.0054	0.0046	0.0060
	Hog	4	0.0250	0.0132	0.0391
Kidney.....	Beef	5	0.0057	0.0041	0.0082
	Hog	4	0.0059	0.0047	0.0075

**Edible Viscera.**—Various other parts of the meat-producing animals, in addition to the carcass meat, are used for food. The iron content of some of these parts has been given in the paragraphs just preceding. A few scattered analyses of older origin are found in the literature. Atwater and Bryant (*U. S. Dept. of Agr., Office of Expt. Stations, Bull. 28* (rev.), 1906) give the composition of certain edible viscera of beef, veal, mutton, and pork.

## PERCENTAGE COMPOSITION OF EDIBLE VISCERA—ATWATER AND BRYANT

		No. of samples	Water	Fat	Protein	Ash	Calories per pound
Beef.....	Brain	1	80.6	9.3	8.9	1.1	555
	Heart	2	62.6	20.4	16.0	1.0	1,160
	Kidney	3	76.7	4.8	16.6	1.2	520
	Liver	6	71.2	4.5	20.4	1.6	605
	Lungs	1	79.7	3.2	16.4	1.0	440
	Marrow	1	3.3	92.8	2.2	1.3	3,955
	Sweetbreads	1	70.9	12.1	16.8	1.6	825
	Suet	9	13.7	81.8	4.7	0.3	3,540
	Tongue	3	70.8	9.2	18.9	1.0	740
Veal.....	Heart	1	73.2	9.6	16.8	1.0	720
	Kidneys	2	75.8	6.4	16.9	1.3	585
	Liver	2	73.0	5.3	19.0	1.3	575
	Lungs	1	76.8	5.0	17.1	1.1	530
Mutton.....	Heart	2	69.5	12.6	16.9	0.9	845
	Kidneys	1	78.7	3.2	16.5	1.3	440
	Kidney fat	2	3.4	95.4	1.8	0.1	4,060
	Liver	2	61.2	9.0	23.1	1.7	905
	Lungs	2	75.9	2.8	20.2	1.2	495
Pork.....	Brains	1	75.8	10.3	11.7	1.6	655
	Heart	1	75.6	6.3	17.1	1.0	585
	Kidneys	2	77.8	4.8	15.5	1.2	490
	Liver	1	71.4	4.5	21.3	1.4	615
	Lungs	1	83.3	4.0	11.9	0.9	390
	Marrow	6	14.6	81.2	2.3	...	3,470
	Skin	7	46.3	22.7	26.4	0.6	1,450

Powick and Høagland (*J. Agr. Research* 1924, 28, 339) report the composition of a number of tissue analysed by them. A summary of their results is given herewith.

## COMPOSITION OF LIVERS

Material	Number of samples	Moisture	Ether extract	Total nitrogen	Purine nitrogen	Total creatinine nitrogen	Glycogen	Dextrose	Total carbohydrate as dextrose
		%	%	%	%	%	%	%	%
Ox liver.....	9	70.05	2.98	3.26	0.123	0.014	1.05	2.22	3.53
Calf liver.....	6	69.83	5.41	3.15	0.142	0.015	0.49	1.04	1.57
Hog liver.....	6	72.84	5.28	3.17	0.164	.....	0.00	0.25	0.25

Material	Number of samples	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Copper	Iron	Calcium	Magnesium	Potassium	Sodium
		%	%	%	%	%	%	%	%	%
Ox liver..	9	0.358	0.099	0.259	0.0039	0.0056	0.0050	0.019	0.281	0.086
Calf liver..	6	0.361	0.099	0.262	0.0045	.....	0.0059	0.020	0.311	0.082
Hog liver..	6	0.369	0.097	0.279	0.0004	.....	0.0081	0.021	0.296	0.090

<sup>1</sup> Average of 4 analyses. <sup>2</sup> Average of 3 analyses. <sup>3</sup> Average of 2 analyses.

COMPOSITION OF VARIOUS EDIBLE VISCERA OTHER THAN LIVER

Material	Number of samples	Number of organs making up composite sample	Moisture %	Ether extract %	Total nitrogen %	Purine nitrogen %	Total phos- phorus %	Inorganic phos- phorus %	Organic phos- phorus %	Ash %
Ox heart.....	6	10	71.72	9.77	2.77	0.085	0.201	0.001	0.140	.....
Lamb heart.....	1 (composite)	10	79.29	1.63	2.83	0.082	0.209	0.075	0.134	.....
Ox brains.....	2 (composite)	10	77.65	7.03	2.20	0.086	0.171	0.044	0.127	.....
Hog brains.....	2 (composite)	10 and 22	78.36	7.48	1.71	0.032	0.345	0.059	0.286	1.53
Sheep brains.....	1 (composite)	50 and 50	78.26	7.48	1.65	0.038	0.342	0.076	0.266	1.45
Beef kidneys.....	1 (composite)	70	80.12	2.07	1.71	0.040	0.333	0.071	0.262	1.38
Hog kidneys.....	1 (composite)	70	76.25	5.41	2.52	0.083	0.230	0.090	0.140	1.11
Ox tongue.....	2	60	76.25	5.41	2.72	0.095	0.204	0.103	0.101	1.18
Hog tongue.....	1	14	68.30	13.27	2.89	0.077	0.164	0.091	0.073	0.92
Lamb tongue.....	1	46	69.51	15.32	2.72	0.081	0.186	0.095	0.091	0.94
Calf pancreas.....	1	35	70.44	11.10	2.23	0.088	0.147	0.078	0.069	0.80
Hog pancreas.....	1	Unknown	64.12	18.40	2.63	0.206	0.326	0.063	0.263	1.25
Ox spleens.....	1	5	77.48	2.26	2.54	0.126	0.331	0.067	0.264	1.19
Hog spleens.....	1	50	79.00	1.88	2.94	0.180	0.284	0.059	0.225	1.41
Ox lungs.....	1	50	78.17	2.07	2.76	0.167	0.298	0.078	0.220	1.44
Lamb lungs.....	2	50	78.00	2.36	2.89	0.109	0.215	0.046	0.169	1.09
Ox stomach, cooked.....	1	5 pairs	81.08	2.39	2.90	0.133	0.180	0.075	0.105	1.30
Hog stomach, cooked.....	1	.....	73.96	8.95	2.65	0.074	0.040	0.003	0.037	0.22
					2.64	0.074	0.118	0.020	0.098	0.57



Wright and Forsyth (*New Zealand J. Science and Techn.*, 1926, 8, 305; *J. Soc. Chem. Ind.*, 1927, 46, 36 T.) report the chemical composition of the edible viscera of sheep and oxen.

#### PERCENTAGE COMPOSITION OF EDIBLE VISCERA FROM SHEEP

	Tongue	Heart	Kidney	Liver	Brain	Lamb thymus	Diaphragm	Blood
Water.....	69.46	69.80	78.96	71.26	80.24	79.46	73.24	80.66
Fat.....	14.71	11.54	3.52	3.90	6.92	3.84	2.78	0.58
Ash.....	1.21	1.12	1.20	1.22	1.30	1.27	1.02	0.81
Phosphorus.....	0.19	0.20	0.22	0.33	0.29	0.22	0.21	0.02
Protein.....	14.50	16.88	15.88	19.38	10.38	14.13	22.13	17.44
Total nitrogen.....	2.32	2.70	2.54	3.10	1.66	2.26	3.54	2.79
Insol. nitrogen.....	1.66	1.62	1.34	2.23	1.47	1.73	2.37	.....
Amino nitrogen.....	0.22	0.24	0.21	0.26	0.08	0.34	0.28	.....
Carbohydrate, as dextrose.....	0.26	0.26	0.36	.....	.....	.....	0.19	.....
Acidity as lactic acid.....	0.17	0.40	0.22	.....	.....	.....	0.38	.....

#### PERCENTAGE COMPOSITION OF EDIBLE VISCERA FROM OXEN

	Tongue	Heart	Kidney	Liver	Brain	Pancreas	Tripe	Blood
Water.....	68.30	70.32	70.36	69.82	78.04	70.92	84.72	79.34
Fat.....	11.46	10.86	2.78	3.12	7.20	12.16	1.96	0.52
Ash.....	1.18	1.24	1.16	1.32	1.44	1.54	0.26	0.90
Phosphorus.....	0.19	0.19	0.23	0.36	0.34	0.42	0.05	0.02
Protein.....	18.13	16.93	16.25	20.38	11.13	17.06	12.56	19.19
Total nitrogen.....	2.90	2.66	2.60	3.26	1.78	2.73	2.01	3.07
Amino nitrogen.....	0.34	0.26	0.38	0.24	.....	1.881	1.871	.....
Carbohydrate, as dextrose.....	0.17	0.51	0.30	.....	.....	.....	.....	.....

<sup>1</sup> These values are too high for amino nitrogen and are probably intended for insoluble nitrogen.

#### PERCENTAGE COMPOSITION OF EDIBLE VISCERA OF BEEF CATTLE

Tissue	Age	Fatness	Number of samples	Water	Fat	Protein	Ash	Water-to-protein ratio
Tongue.....			8	64.00	19.15	15.20	0.80	4.2
Lean heart.....			7	77.40	4.05	16.60	1.04	4.7
Fat heart.....			1	65.83	19.45	13.30	0.84	4.9
Stomachs.....			4	77.40	9.76	10.75	0.95	7.2
Thymus.....	Veal 3 mo.		3	78.03	5.03	16.60	1.84	4.7
	Veal 6 mo.		3	69.18	15.50	15.58	1.56	4.4
	Baby beef 11 mo.		3	68.02	16.40	14.31	1.54	4.8
	Fully grown	Thin	4	64.02	20.40	14.35	1.45	4.4
		Medium	3	59.39	36.92	11.61	1.07	4.3
		Fat	4	39.29	51.53	8.16	0.76	4.8
Pancreas.....	Veal		9	71.88	10.32	15.51	1.38	4.6
	Baby beef	Thin	1	72.20	9.61	15.88	1.46	4.5
	Baby beef	Medium	1	69.66	11.65	16.06	1.47	4.3
	Baby beef	Fat	1	59.92	23.45	12.75	1.19	4.7
	Fully grown	Thin	4	61.69	21.05	14.74	1.21	4.2
	Fully grown	Medium	3	58.10	24.82	13.56	1.19	4.3
	Fully grown	Fat	4	51.93	33.30	11.82	1.08	4.4
Spleen.....	All ages		23	77.07	2.40	18.25	1.39	4.2
Kidney.....	All ages		29	75.21	7.53	15.08	1.11	5.0
Liver.....	All ages		38	69.92	2.96	19.39	1.43	3.6

Moulton (*Meat through the Microscope*, University of Chicago Press, Chicago, 1929, p. 312), in discussing the manufacture of sausage and the water-to-protein ratio in sausage materials and other meats, presents a good many analyses of edible viscera, partly based on the work of Moulton, Trowbridge, and Haigh, previously referred to. The accompanying tables give the composition of certain edible viscera and fatty tissues of the beef animal. As a rule age and fatness have but a relatively minor effect upon the composition of the tongue, marketable heart, stomachs, spleen, kidneys, and liver. But other tissues, such as the thymus, pancreas,

PERCENTAGE COMPOSITION OF FATTY TISSUES OF BEEF CATTLE

Tissue	Age	Fatness	Number of samples	Water	Fat	Protein	Ash	Water-to-protein ratio
Thoracic fat.....	All ages		8	16.80	79.46	3.20	0.23	5.3
Offal fat.....	Veal	Thin	3	52.95	37.12	9.52	0.67	5.6
		Medium	3	30.34	64.44	5.65	0.44	5.4
		Fat	3	21.54	74.55	4.29	0.31	5.0
	Beef	Emaciated	1	81.60	5.03	12.00	1.06	6.8
		Very thin	2	35.13	57.42	6.16	0.46	5.7
		Thin	8	19.14	76.95	3.98	0.23	4.8
		Medium	11	14.15	82.93	2.71	0.20	5.2
		Fat	12	8.94	89.13	1.82	0.12	4.9
Kidney fat.....	Veal	Thin	3	26.34	65.60	7.36	0.44	3.6
		Medium	3	13.97	83.28	3.61	0.28	3.9
		Fat	3	10.51	86.83	2.65	0.22	4.0
	Beef	Emaciated	1	81.42	4.59	9.63	1.21	8.5
		Very thin	2	23.29	72.76	5.91	0.31	3.9
		Thin to fat	31	6.22	91.68	2.00	0.13	3.1

offal fat, and kidney fat are affected by both age and fatness of the animal. The ratio of water to protein in these tissues is given here without comment, but it will be referred to under the general subject of sausage.

### The Structure of Meat

The lean of meat, or flesh in the restricted sense of muscular contractile tissue, consists anatomically of numerous small, thread-like fibres, the muscular fibres lying side by side and united and supported by the connective tissue. The connective tissue carries the blood-vessels and nerves and contains among its constituents the gelatin-forming substance, collagen, other proteins such as globulin and albumin, and fat. The muscle fibres themselves consist of a delicate structureless covering membrane corresponding to a

cell wall, the *sarcolemma*, composed of a protein similar to elastin, and the enclosed sarcoplasm, or muscle plasma.

Microscopically, three sorts of muscle fibres are distinguished, corresponding to voluntary or striated, smooth and heart muscle. The first class, the skeletal muscle, is by far the most important and abundant in the animal body. It comprises the muscles attached to the skeleton. Since it forms the major part of meat food products, it is the one of chief interest to the food chemist and analyst. Under the microscope it appears to be formed of numerous parallel cylindrical fibres about 0.05 mm., or  $\frac{1}{500}$  inch, in diameter and about 18 to 36 mm., or  $\frac{3}{4}$  to  $1\frac{1}{2}$  inch, long. A single fibre, then, is about 500 times as long as it is wide. A diagrammatic view of such a fibre is shown in Figure 12, A and B giving side views and C a cross sectional view. The characteristic cross striations, consisting of alternate dark and light bands and, under certain conditions, fine longitudinal markings are visible under moderately high powers. The striated muscle fibres are polynuclear and each contains, just below the sarcolemma, numerous oval nuclei. These various structures are shown more prominently by proper staining as by eosin and haematoxylin.

Still finer structures are observable by more minute microscopic examination aided by appropriate

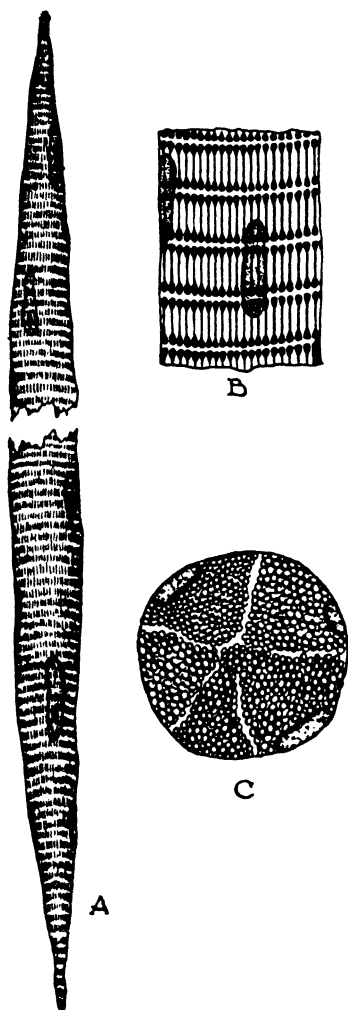


FIG. 12.—Diagrammatic representation of a muscle fibre. A, Muscle fibre, cylindrical in shape,  $\frac{1}{500}$  inch in diameter, and often an inch or more in length. B, Longitudinal section, very highly magnified. C, Cross-section, very highly magnified.

chemical treatment. Each muscle fibre is seen to consist of minute fibrillae (sarcostyles) which can be split transversely into numerous discs (sarcous elements). Compare Figures 13 to 19.

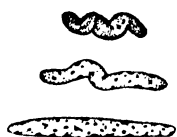


FIG. 13.—Nuclei of smooth muscle fibres from the artery of a dog. (Stöhr.)

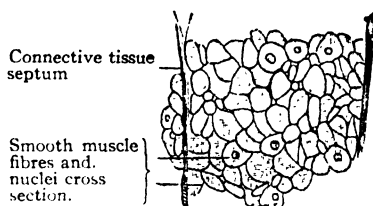


FIG. 14.—Section of the circular muscle coat of the human intestine.  $\times 560$ . (Stöhr.)

Macroscopically the striated muscular fibres are bound together by connective tissues successively into fasciculi, bundles and muscles.

Smooth or non-striated muscle is found principally in the gastro-intestinal tract, the blood vessels, genito-urinary tract, and skin. It consists of spindle-shaped cells, about  $\frac{1}{10}$  by  $\frac{1}{200}$  mm., each of which contains a central rod-shaped nucleus. Fine longitudinal markings can sometimes be observed.

Heart muscle occurs only in the heart. It consists of short, cylindrical, branching cells, attached end to end. Each cell contains a single oval nucleus. The cells show finer cross-striations than striated muscle and also fine longitudinal markings.

Moran and Smith (Dept. of Scientific and Industrial Research, Food Investigation, *Special Report No. 36*, London, 1929) in their discussion of the post-mortem changes in animal tissues, present some excellent photomicrographs of muscle tissue.

The history and chemistry of red and white muscle has been reviewed by Needham (*Physiol. Reviews*, 1926, 6, 1-27).



FIG. 15.—Muscle fibre of a frog.  $\times 240$ . *f*, Fibrillae; *k*, nucleus. (Stöhr.)



FIG. 16.—Fibrils from the wing muscles of a wasp. (Schafer.) A, Contracted; B, stretched; C, Uncontracted. The dark bands are bisected by the light stripes, but they do not show the median membranes.

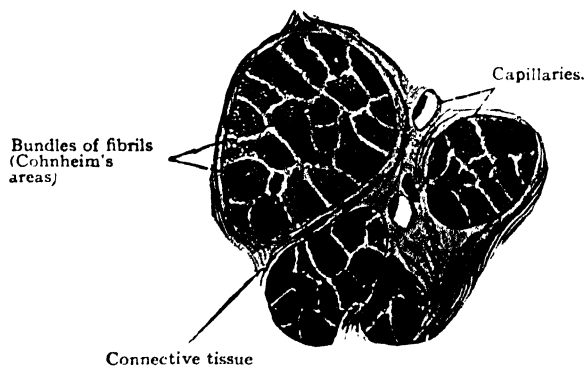


FIG. 17.—Cross-section of four muscle fibres of the human vocal muscle.  $\times 590$ . (Stöhr.)

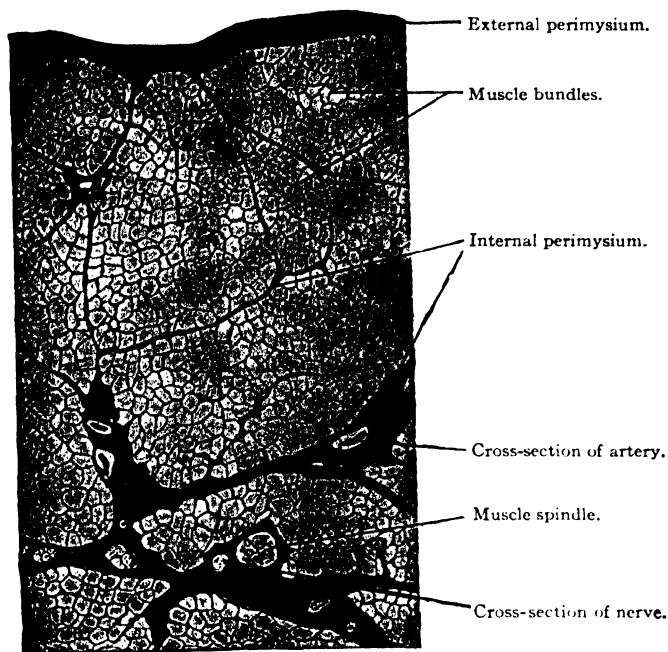


FIG. 18.—From a cross-section of the omohyoid muscle of man.  $\times 60$ . (Stohr.)

### Identification of Species

An experienced person can distinguish the meats of the principal food animals, fish, crustaceans, shell-fish, etc., by their appearance, taste, and smell. It would be difficult, however, to describe these differences accurately. The works on meat inspection treat of these matters in some detail. The percentage of glycogen, in many instances, affords means for the detection of horse-flesh. The constants of the fats associated with meats, such as iodine value, saponification value, refractive index, "titre" of fatty acids, etc., also afford chemical means of identification in many cases, where the meat is unmixed. For the constants of fats see

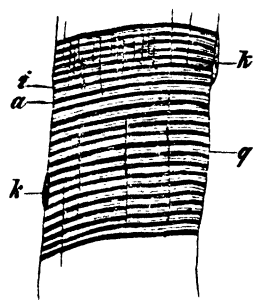


FIG. 19.—Part of a longitudinal view of a human striated muscle fibre. (Stohr.) *a*, Anisotropic; *i*, isotropic band; *k*, nucleus; *g*, ground membrane.  $\times 560$ .

Vol. II. The analyst of meat food products should be familiar with all kinds of meats and meat-food products, from the physical and edible standpoint, as well as from the analytical standpoint.

**The Precipitin Method.**—The fact that more or less specific precipitins can be developed in the blood of one animal by the repeated or periodic injection of the blood serum from another species of animal, has been proposed as the basis of a method for distinguishing different kinds of meat and for detecting the kinds of meat in a mixture of meats such as sausage. It has been proposed especially as a means for detecting horse meat in sausage, although it can be used for the detection of meat from other species equally well. In brief, the method consists in injecting periodically into rabbits, blood or serum from the species for which an antiserum is sought, and after a time preparing the antiserum from the treated rabbits. An extract is then made of the sample and tested *in vitro* with the specific antiserum. Naturally, a separate antiserum is generally necessary for each species or variety; however, in some cases the same antiserum acts on more than one of several closely related species or varieties.

**General Technique.**—In order to prepare the antisera a completely equipped bacteriological laboratory with large experimental animals and a skilled operator are necessary, and this limits the usefulness of the method. Until the specific antisera can be readily purchased, the method is necessarily greatly restricted in its application and could be used only in important cases and possibly only in certain legal cases. With reliable antisera available, the method could be applied by the ordinary chemist; otherwise he would be precluded from using it. Only the large serum establishments are in a position to prepare the antisera, and without special demand for the sera they would naturally not be prepared except for scientific experiments.

**Preparation of the Antiserum.**—The special technique for the serum preparation can be only briefly described here. It must be learned under a competent instructor at a properly equipped establishment and requires skill derived from much experience. Rabbits are the experimental animals commonly used. They are injected with defibrinated blood or blood serum from the species for which an antiserum is desired, at 4 or 5 days' intervals, 8 to 15 c.c. being used intraperitoneally or 3 to 8 c.c. by the intravenous method. It is best to work with 5 or 6 rabbits at one time, but the serum from

each rabbit is kept separate. When the intraperitoneal method is used there is usually no serious reaction, but with the intravenous method the animals sometimes become sick or die. At intervals the blood is tested for its activity by withdrawing 5 c.c. from the large vein of the ear, centrifuging it and working with the clear serum. When the blood is sufficiently active, and usually 7 to 10 days after the last injection, the animal is bled and the clear serum separated after standing 24 hours. A good antiserum should be perfectly clear (not opalescent) and should be highly active. If opalescent it may be clarified by filtration through unglazed porcelain or a layer of kieselguhr on an ordinary filter, but it is best to prepare the serum in such a way that it is clear at the start. It is best to preserve the serum by low temperatures ( $8^{\circ}$  to  $10^{\circ}$ ) rather than by antiseptics such as phenol or chloroform. The activity of antisera is measured by determining the dilution of blood in which a precipitate is produced. Thus dilutions are made in 0.85% sodium chloride solutions of 1:500, 1:1000, 1:10,000, 1:20,000, etc., and to 1 c.c. of the diluted blood 0.1 c.c. antiserum is added. The cloud usually appears in 1 to 5 minutes. Very strong and very weak antisera are both likely to cause errors, the strong by producing precipitates in the case of related or remotely related species, and the weak through failure or delay in producing the precipitate. The strong antisera may be weakened with salt solution before use.

*Testing for Horseflesh.*—The method of testing for horseflesh is typical, and the description, with slight or no modification, will apply to other meats as well. Large pieces of meat can usually be identified by inspection, and hence the precipitin method finds its chief application, in food analysis, in the case of comminuted meats such as sausage in which, on the Continent of Europe, horseflesh is sometimes used as an adulterant. The method is ordinarily not applicable to any but raw meats; however, reactions have been obtained with incompletely cooked meat, uncooked cured ham, and old, dry, smoked but uncooked sausage (Nötel, *Z. Hyg.*, 1902, **39**, 373-8; Uhlenhuth, *Deutsche med. Wochenschr.*, **27**, 409-501). Schmidt (*Z. Immunität*, **13**, 166-85) obtained an antiserum by means of blood serum coagulated at  $70^{\circ}$  then dissolved in dilute sodium hydroxide solution, which reacted with similar but not with normal serum, but no success attended experiments with sodium hydroxide solutions of coagulated muscle proteins. Therefore, for the present at least, the



method is, for the most part, applicable only to raw meats. The following methods are compiled for Nuttall, *Blood Immunity and Relationship*; König, *Nahrungs and Genussmittel*, Vol. 3; and various sources in recent literature to which references are given and to which the reader is referred for more detailed information.

**Method for Whole Pieces of Meat.**—While large pieces of meat can generally be identified by inspection, occasion may arise when inspection may leave a doubt as to the true identity. By the use of sterile knives 30 to 40 grm. of lean meat are removed from the interior of the piece to a sterilised Erlenmeyer flask containing 100 c.c. of sterile 0.85% sodium chloride solution. The extraction proceeds with fresh meat 1 to 3 hours at room temperature or overnight in the ice-box. With smoked, pickled, or decomposed meat the extraction is longer—even to 24 hours. Very salty meat can be freshened by immersion in sterilised distilled water before extraction. Agitation is to be avoided during the extraction period. The extract is generally usable when it foams on shaking in a test-tube. The extract is clarified by filtration through ignited kieselguhr, stirred up in a sterile salt solution, and poured over a hard filter on a Büchner funnel or through unglazed porcelain. The test is carried out by means of sterile glassware throughout. The various test-tubes contain:

No. 1, 1 c.c. extract to be tested.

No. 2, 1 c.c. extract to be tested.

No. 3, 1 c.c. known extract of horse meat.

No. 4, 1 c.c. known extract of beef.

No. 5, 1 c.c. known extract of pork.

No. 6, 1 c.c. sterile 0.85% sodium chloride solution.

All the extracts are prepared as directed for horse meat. To each tube, with the exception of No. 2, is added 0.1 c.c. clear, active horse antiserum in such a way that it runs down the sides of the tubes to the bottom without mixing. Tube No. 2 is treated in the same way with 0.1 c.c. normal rabbit-serum. A cloudiness should be obtained in 1–2 minutes in tube 3 and also in 1 if the meat is in fact horse meat. In 5 minutes this cloudiness increases and in 10 minutes longer settles to the bottom as a precipitate. No indications after 2 minutes are to be considered.

**Method for Comminuted Meat, Sausage.**—The sample is taken from the centre of the piece or mass and extracted as above. Or

is rubbed in a mortar and then extracted. Sometimes it is advisable to extract first with ether. Uhlenhuth in some cases recommends squeezing through cheesecloth or similar material and adding the press-juice to the extract and finally filtering as before. The extraction of sausage and ground meats is much more difficult than a single lean piece, and the operator must be guided accordingly. The test proceeds as stated above, except that, on account of the smaller amount of horse meat present, a very active serum is necessary (1:20,000 is active enough to detect 5% addition of horse meat). In addition to the test-tubes enumerated above, two are added containing, respectively, extracts of beef and horse sausage.

*The specificity of antisera* is not absolute in all cases. Uhlenhuth obtained a positive reaction with antisera for pig, sheep, horse, donkey, and cat blood, when these were tested on the corresponding meats. However, the anti-sheep serum gave almost as great a reaction with goat's meat as with sheep's and less with beef extract. A similar fact is that the blood of various species of monkeys reacts with anti-human serum, but to a less extent than human blood. Very active antisera are less specific than average, and tests in dilute solution are more specific than in concentrated, although there is a fairly definite limit to the dilution which is practicable. Closely related species, such as horse and ass, and dog and fox, are difficult or impossible to distinguish by the method.

*The precipitate*, formed between precipitin and antigen, is derived principally from the antiserum. Thus Welch and Chapman (*Z. Immunität*, **9**, 517-29; *J. Hyg.*, **10**, 177-83) consider it incorrect to speak of coagulation of antigen or to look upon the antigen as the precipitable substance. When the precipitation is complete the weight of the precipitate is independent of the amount of antigen, but with partial precipitation the weight of the precipitate is determined by the amount of antigen.

For further discussion of the precipitin method in food analysis and legal cases the chemist may consult Saint-Sernin, *Biological Methods for Distinguishing the Various Kinds of Meat*, *Ann. fals.*, Vol. X, **4**, 334-8; Schmidt, *Cairo Sci. J.*, **5**, 271-89. (See also p. 450.)

Rosenberg (*Centr. Bakt. Parasitenk* I Abt, 1928, **107**, 448) has pointed out that the precipitins from unheated meat are different from those produced by heated meat. Hence, rabbits must be injected with extracts of cooked meat if one wishes to detect horse flesh in cooked meats.

Cesari (*Ann. chim. anal. chim. appl.*, 1924, 6, 169 and 203) discusses the methods which can be used to identify horse meat in food products. The theory of antigen, antibody, and complement is discussed in an elementary way, so that a chemist who is ignorant of biology can understand it without difficulty. He shows how suitable blood serum may be developed for use as a reagent in the examination of meat extracts. Then follow complete directions for preparing suitable serums by inoculating rabbits with serum from different animals, for the preparation of the antibodies from such things as sausages and for carrying out the coagulation tests. Fally's method of determining the deviation of the complement is also described and the results are given for a series of tests made with various food products, some of which proved to contain horse-meat.

For other methods bearing on the identification of species see pages 450 and 523.

### Constituents of Meat

Lean meat, that is, muscular tissue, with all the prominent connective tissue with the contained fat removed, contains approximately 75% water and 25% solids in all vertebrate animals. Of the solids, approximately 80% are proteins and the remaining 20% consist of various water-soluble organic substances conveniently called "extractives," lipoids including some true fat, and inorganic salts. Moulton, Trowbridge, and Haigh (*Missouri Agr. Expt. Sta. Research Bull.* 55, 1922, 25) have shown that the lean flesh of beef cattle calculated to a fat-free basis contains 77% water, 21.88% protein (nitrogen x 6.25) and 1.0 to 1.1% ash.

Among the early investigators of this question are Petersen (*Z. f. Biol.*, 1871, 7, 166), Huppert (*Z. f. Biol.*, 1871, 7, 354), Voit, Grouven, Stohmann, and Seegen (*Wiener Akademie-Anzeigen*, 1870, 230). These investigators selected animals that were not fat, removed the fatty tissue and tendon as completely as possible, and prevented loss of water by care and speed in working or by enclosing the fresh flesh in a glass vessel. The fat, when determined, was extracted by ether, and the nitrogen was determined by the old Will-Varrentrapp method, the accuracy of which may be questioned. Their results are given in the accompanying table. The average figure given by Grouven for meat was obtained from a number of animals, including some fat ones. It is worth while comparing the figure

for nitrogen in the fat-free flesh of cattle given by Moulton, Trow-  
 PERCENTAGE COMPOSITION OF SAMPLES OF LEAN FLESH—OLDER  
 WORK

Investigator	Number of samples	Kind of flesh	Water	Nitro- gen	Fat
Peterson.....	2	Cattle fore leg	77.69	3.29	0.81
	2	Cattle hind leg	75.48	3.30	3.20
	2	Swine fore leg	75.52	3.23	3.76
	2	Swine hind leg	72.96	3.27	5.60
	4	Sheep	76.67	3.15	2.82
	4	Calf	78.85	3.18	0.82
	2	Horse A	73.38	3.59	1.85
	2	Horse B	76.00	3.37	0.93
Voit.....	.....	Market meat	75.79	3.40	.....
Grouven.....	.....	Meat	74.70	.....	.....
Stohmann.....	.....	Goat	.....	3.33	.....
	.....	Lamb	.....	3.52	.....
	.....	Horse	.....	3.40	.....
Seegen.....	.....	Flesh	.....	3.40+	.....
Huppert.....	17	Cattle market meat	74.48	3.322	.....
	22	Cattle direct from slaughter	76.11	3.285	.....

bridge and Haigh with the figures obtained by these early investigators. The former found 3.5% nitrogen ( $21.88 \div 6.25$ ). When calculated to the fat-free basis, the older figures will average a bit lower than this.

Among the more recent investigators are Powick and Hoagland, who report (*J. Agr. Research*, 1924, **28**, 339) the following composition of lean beef muscle.

PERCENTAGE COMPOSITION OF LEAN BEEF MUSCLE—POWICK  
 AND HOAGLAND

Kind of meat	Number of samples	Water	Fat	Nitro- gen	Ash	Phosphorus	
						Total	Inor- ganic
Beef round.....	8	74.33	2.82	3.41	1.07	0.203	0.109
Beef rump.....	7	73.01	4.68	3.28	1.03	0.105	0.102
Beef loin.....	7	72.07	6.00	3.27	1.00	0.188	0.098

Feder (*Z. Unters. Nahr. Genussm.*, 1913, **25**, 577; 1922, **43**, 193), in connection with his studies on the determination of added water in sausage and similar meats, has reported the percentage composition of emaciated flesh. His results follow:

	Water	Fat	Ash	Non-fatty organic matter	Ratio
Shoulder muscle, 7-year-old cow....	78.95	1.33	1.14	18.58	4.25
Composite flesh, badly emaciated....	80.78	0.35	0.98	17.89	4.52

Hoagland and Powick (*J. Agr. Research*, 1925, 31, 1001) have reported the percentage composition of the flesh of twenty extremely emaciated cattle and twelve very thin cattle.

		Water	Fat	Protein	Ash	Ratio
20 emaciated cattle.....	Maximum	82.11	1.13	20.38	1.08	4.7
	Minimum	77.38	0.21	17.31	0.88	3.8
	Average	80.09	0.45	18.90	0.99	4.2
12 very thin cattle.....	Maximum	80.49	2.02	21.06	1.10	4.4
	Minimum	76.81	0.36	18.13	0.93	3.6
	Average	78.84	0.75	19.65	1.03	4.0

The results reported from the Missouri Agricultural Experiment Station on cattle include much that is useful here. The author has prepared the accompanying table from those results.

PERCENTAGE COMPOSITION OF LEAN FLESH OF BEEF CATTLE—  
MISSOURI RESULTS

Cut of meat	Condition	Number of animals	Water	Fat	Nitro- gen	Pro- tein	Ash
Beef Steers							
Round.....	Thin	9	75.89	2.34	3.193	19.96	1.07
	Medium	10	73.86	4.29	3.272	20.45	1.06
	Fat	11	70.61	7.94	3.168	19.80	1.01
Loin.....	Thin	9	73.55	4.40	3.204	20.04	1.07
	Medium	10	72.04	6.80	3.137	19.61	1.02
	Fat	11	67.29	11.85	3.057	19.11	0.98
Rib.....	Thin	7	71.98	6.93	3.141	19.63	1.01
	Medium	8	69.27	9.81	3.090	19.31	0.96
	Fat	10	62.93	17.87	2.878	17.99	0.86
Composite lean flesh.....	Fat	1	71.31	6.88	3.28	20.50	0.99
	Good	1	71.40	6.21	3.03	18.94	0.98
	Medium	1	71.29	8.54	3.02	18.88	0.93
	Thin	1	73.52	4.91	3.17	19.81	1.00
	Very thin	1	74.24	2.78	3.24	20.25	0.93
	Very, very thin	1	76.37	1.87	3.15	19.69	1.05
Old Cows							
Composite flesh.....	Thin	1	68.70	9.52	3.20	20.00	0.98
	Fat	1	67.30	11.67	3.11	19.44	1.00
	Fat	1	67.12	12.70	3.07	19.19	0.97

An examination of the individual results used in obtaining the average figures presented in the Missouri data shows that the fatness of the lean meat increases in order from round to loin to rib. Also, the amount of fat intimately associated with the lean increases with increasing age and fatness of the cattle.

The gross composition of lean muscle can be represented in tabular fashion as follows.

Muscular tissue	{	Water 75%	{	Proteins 20%
		Solids 25%		Extractives 4%
				Inorganic salts 1%

*Muscle Plasma.*—During the life of an animal the muscle fibre has a semi-fluid consistency. The semi-fluid part is called muscle plasma. The solid part which bounds the fibre is called the sarcolemma, while the fine framework of the fibre which extends into the plasma is called muscle stroma. The proteins held in the semi-fluid state are changed in chemical properties after death during rigor mortis and the changes of autolysis which follow. By pressing the ice-cold, purified muscle of the frog immediately after death under suitable conditions, Kühne obtained the muscle plasma as a syrupy liquid of faintly alkaline reaction, which at ordinary temperature soon clotted after the manner of blood plasma.

According to von Fürth, as cited by Mathews, (*Physiological Chemistry*, Wm. Wood and Co., New York, 1925) the proteins of the plasma are two in number, myosin and myogen. Myosin is a globulin-like protein which coagulates on quick heating to 44–50° C. It coagulates spontaneously on long standing and changes to myosin fibrin. Myosin is soluble in solutions of neutral salts, is precipitated by dialysis and by dilution with water, and is easily salted out of solution by half-saturation with ammonium sulphate. This latter step permits its separation from myogen. It is easily precipitated by acids.

Myogen is an albumin and is present in quantities three or four times as great as the quantity of myosin. Myogen is not precipitated by dialysis nor by half-saturation with ammonium sulphate. It is precipitated by dilute acids like a globulin. It coagulates at 30–40° C., and on standing or agitation coagulates spontaneously into myogen fibrin.

The proteins of the sarcolemma and stroma are usually not soluble in ammonium chloride or other neutral salt used to dissolve myosin. The stroma contains nucleo-proteins.

Saxl states that fresh living mammalian skeletal muscle can be extracted to the amount of 84 to 90% with ammonium chloride solutions, providing rigor is prevented, whereas after rigor for a short time only but 2 to 3% is extractable. Salkowski states that of the total nitrogen of flesh, 77.4% is in the form of insoluble coagulable protein, 10.08% in the soluble coagulable form, and 12.52% in the form of non-protein nitrogen. However, as will be shown later, this latter division of the proteins of dead flesh can be altered within wide limits by varying the temperature and the salt used in the extraction.

The nitrogenous extractives include: creatine, methyl-guanidine, carnosine (also called ignotine), inosine (carnine), carnitine, sarcosine, taurine, glycoll, urea, hypoxanthine, myokinine, neosine, mirge-line, and possibly carnisapidine.

According to Mathews, 1 kilo. of fresh muscle contains the following amounts of certain extractives.

	Horse	Ox	Salmon	Calf	Pig
Creatine.....	0.58	.....	3 2	.....	.....
Purines.....	0.09 -0.07	.....	.....	.....	.....
Carnosine.....	1.82	1.30	0.55	1.76	1.95
Methyl-guanidine.....	0.083-0.1	.....	.....	0.22	.....
Carnitine.....	0.2 -0.17	.....	.....	0.19	0.30

These figures are incomplete and leave much to be desired. Mathews (*loc. cit.*, p. 631) cites Burian and Hall as authority for the following figures for total purine nitrogen in percentage of the wet weight of muscle:

Horse meat.....	0.055
Ox meat.....	0.062
Veal.....	0.071

According to Hammarsten and Hedin (*A Text Book of Physiological Chemistry*, John Wiley and Sons, London, 1914, p. 599) the muscles of mammals and birds have the following percentage composition.

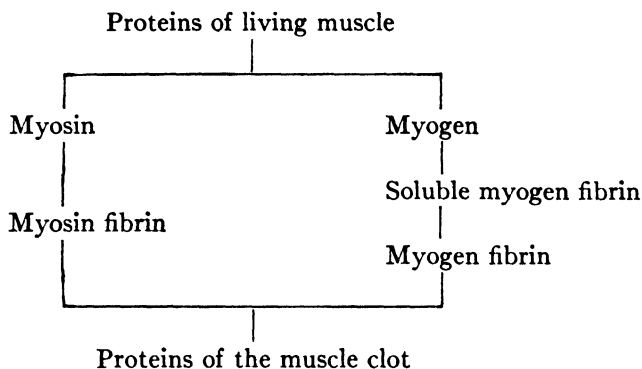
	Muscle of mammals	Muscle of birds
Water.....	72.2 - 78.3	71.7 - 77.3
Organic bodies.....	20.7 - 26.3	21.7 - 26.3
Inorganic bodies.....	1 - 1.5	1 - 1.9
Myosin.....	3 - 10.6	3 - 11
Stroma substance.....	7.8 - 16.1	8.8 - 18.4
Creatine.....	0.2 - 0.45	0.3 - 0.49
Carnosine.....	0.13 - 0.40	
Carnitine.....	0.019	
Purine bases.....	0.13 - 0.17	0.07 - 0.13
Inosinic acid, barium salt.....	0.01	0.01 - 0.03
Phosphocarnic acid.....	0.057 - 0.24	
Inosite.....	0.003	
Glycogen.....	0.1 - 3.7	
Lactic acid.....	0.04 - 0.07	

The following table by Hofmann (*Lehrbuch der Zoochemie*) shows the average composition in parts per 1,000 of the muscles of vertebrate animals:

	Mammals	Birds	Cold-blooded vertebrates
Water.....	745 to 783	717 to 773	800
Organic matters			
Coagulated albumin, sarcolemma, nuclei, vessels.....	145 to 167	150 to 177	?
Alkaline albuminate.....	28.5 to 30.1		
Creatine.....	2.0	3.4	2.3
Xanthine and hypoxanthine.....	0.2		
Taurine.....	0.7 (horse)	0.0	1.1
Inositol.....	0.03		
Glycogen.....	4.1 to 5.0		30 to 50
Lactic acid.....	0.4 to 0.7		
Salts			
Potash.....	3.0 to 3.9		
Soda.....	0.40 to 0.43		
Lime.....	0.16 to 0.18		
Magnesia.....	0.40 to 0.41		
Oxide of iron.....	0.03 to 0.10		
Phosphoric acid.....	3.4 to 4.8		
Sodium chloride.....	0.04 to 0.10		

The relationships between the plasma proteins of living and dead muscle are shown in the following diagram:





According to Mathews there is 3 to 4 times as much myogen as myosin. The names given these proteins by different investigators are various. Myogen is the myosinogen of Halliburton. Myosin has been called musculin and is the paramyosinogen of Halliburton.

The diagram given is only an approximation of the facts, as is shown by the various results which can be obtained by extracting dead muscle with pure water and with various concentrations of different salts. It may be that a mixture of proteins is being dealt with and not two individuals. Przibram (*Beiträge chem. Phys. u. Path.*, 1902, 2, 143) has stated that myosin and myogen are present in vertebrates generally, but that myosin alone is present in invertebrates. In the muscle plasma of fishes is found, in addition to the two principal proteins, a peculiar one, myoprotein, which precipitates on dialysis and with acetic acid, but does not coagulate when heated. Myogen in coagulating to myogen fibrin passes through an intermediate stage known as soluble myogen fibrin, whereas myosin passes directly to myosin fibrin. The coagulation of the proteins of living muscle upon the death of an animal is coincident with the setting in of the rigid condition known as rigor mortis.

The voluntary muscles of mammals are commonly classified into two varieties which are known respectively as red muscle and pale muscle, depending on the depth of red colour which they possess. Prominent examples of pale muscles are the white meat of domestic fowl and of rabbit. The red colour of muscles has been shown to be due to oxyhaemoglobin dissolved in the muscle plasma. The presence of other colouring matters in muscle, possibly derivatives

of haemoglobin, has been claimed by some observers and disputed by others as having been produced by the reagents used in the separation.

The term nucleo-protein or nuclein is applied to a large group of related substances found in cell nuclei. They are not quantitatively of great importance in muscular tissue. They contain considerable phosphorus and some sulphur. By hydrolysis with alkalies they yield proteins and nucleic acids. With boiling acids the true nucleins yield phosphoric acid and the xanthine bases. They are insoluble in water, alcohol, and ether, but usually dissolve in alkalies.

### Classification of Proteins

It is both convenient and desirable when dealing with the various proteins found in animal and vegetable matter to have some classification which will aid in distinguishing or identifying the different proteins. The following classification is that of the American Society of Biological Chemists as cited by Hawk and Bergeim (*Practical Physiological Chemistry*, P. Blakiston's Son and Co., Inc. Philadelphia, 1931).

#### I. SIMPLE PROTEINS

Protein substances which yield only  $\alpha$ -amino acids or their derivatives on hydrolysis.

**Albumins**, soluble in water and coagulable by heat. Examples: ovalbumin, myogen, and serum albumin.

**Globulins**, insoluble in pure water but soluble in solutions of neutral salts of strong bases with strong acids. Examples: myosin, serum globulin, and ovoglobulin.

**Glutelins**, insoluble in all neutral solvents but soluble in very dilute acids and alkalies. Meats furnish no examples of this class of proteins.

**Alcohol-soluble proteins (prolamins)**, soluble in 70–80% alcohol, insoluble in pure water, absolute alcohol, and other neutral solvents. Meats furnish no examples of this class of proteins.

**Albuminoids** show pronounced insolubility in all neutral reagents. Examples: elastin, collagen, the so-called ossein of bone which is collagen, and keratin.

**Histones**, soluble in water and insoluble in very dilute ammonia and, in the absence of ammonium salts, insoluble even in an excess

of ammonia. These proteins yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield a large number of amino acids, among which the basic ones predominate. Examples: thymus histone, globin, scombrone.

**Protamines** are simpler polypeptides than the proteins listed above. They are soluble in water, uncoagulable by heat, have the property of precipitating aqueous solutions of other proteins, and possess strong basic properties. On hydrolysis they yield comparatively few amino acids, among which the basic ones predominate. Examples (from fish): salmine, sturine, and scombrine.

## II. CONJUGATED PROTEINS

Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

**Nucleoproteins.**—Compounds of one or more protein molecules with nucleic acid. Examples: nucleohistone and cytoglobulin.

**Glycoproteins.**—Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Examples: mucins and mucoids (osseomucoid, tendomucoid).

**Phosphoproteins.**—Compounds of the protein molecule with some, as yet undefined, phosphorus-containing substance other than a nucleic acid or lecithin. Examples: caseinogen and vitellin.

**Hæmoglobins.**—Compounds of the protein molecule with hematin or some similar substance. Examples: hæmoglobin and hæmocyanin.

**Lecithoproteins.**—Compounds of the protein molecule with lecithins. Examples: lecithans and phosphatides.

## III. DERIVED PROTEINS

### 1. Primary Protein Derivatives

Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alteration of the protein molecule.

**Proteans.**—Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes. Examples: myosan and edestan.

**Metaproteins.**—Products of the further action of acids and alkalis, whereby the molecule is so far altered as to form products soluble in very weak acids or alkalis but insoluble in neutral fluids. Examples: acid metaprotein (acid albuminate) and alkali metaprotein (alkali albuminate).

**Coagulated Proteins.**—Insoluble products which result from the action of heat on their solutions or the action of alcohol on the protein.

## 2. Secondary Protein Derivatives

Products of the further hydrolytic cleavage of the protein molecule.

**Proteoses.**—Soluble in water, non-coagulable by heat, and precipitated by saturating their solutions with ammonium or zinc sulphate.

**Peptones.**—Soluble in water, non-coagulable by heat, and not precipitated by saturating their solutions with ammonium sulphate.

**Peptides.**—Definitely characterised combinations of two or more amino acids, the carboxyl group of one being united with the amino group of the other with the elimination of a molecule of water.

Further action on secondary protein derivatives by enzymes or other agent of decomposition will, of course, yield the amino acids and finally carbon dioxide, ammonia, and hydrogen sulphide.

## Muscle Extractives

The heterogeneous group of organic substances known collectively as “extractives” of muscle may be divided into non-nitrogenous and nitrogenous extractives. Some of them, although present in flesh foods in small quantity, are of considerable importance to the analyst.

The non-nitrogenous extractives include: glycogen, dextrin, dextrose or other sugars, lactic acids, and inositol.

The nitrogenous extractives include: creatine, creatinine, methyl guanidine, carnosine (ignotine), inosine (carnine), sarcosine, carnitine, taurine, xanthine, hypoxanthine, myokinine, neosine, mirgeline, carnisapidine, inosinic acid, glycocoll, urea and uric acid.

Fat is sometimes included under non-nitrogenous extractives, but it can best be treated separately. However, it is always found intimately associated with muscular tissue. Many of these substances are brought into solution when meat is extracted with boiling water and hence occur in the various meat extracts which are found on the market. (See **Meat Extracts**—*Creatinine* and *Creatine*, page

504; also Vol. VIII, p. 356.) The nitrogenous extractives are intimately associated with protein metabolism and are of greater importance to the physiologist than to the analyst. Only those extractives which are relatively important from the analytical standpoint will be considered specifically.

**Non-nitrogenous Extractives.**—Glycogen ( $C_6H_{10}O_5$ )<sub>n</sub>, together with its hydrolytic products, dextrin, maltose, and dextrose, is constantly present in muscular tissue in small quantity. As the sugars are constantly consumed in metabolic processes, it follows that glycogen is constantly forming. It is not uniformly distributed through the musculature and is present in certain organs—notably the liver—in greater quantity than in others. Neither is it present in the same proportion in different animals, and since among the large food-animals it is present in horse meat to a greater extent than in other meats, its estimation has been used as the basis of a method for the detection of horse-flesh. Glycogen is a white, amorphous, odourless and tasteless substance, as prepared from animal tissues, of which the liver of the dog is one of the most suitable (Abderhalden, *Biochemischen Arbeitsmethoden* 2, 162). It gives an opalescent solution with water, can be boiled with strong potassium hydroxide solution without decomposition, and is hydrolysed by mineral acids and diastase into dextrose.

Glycogen is strongly dextrorotatory, the value of  $[\alpha]_D$  being +196.6.

Glycogen does not reduce Fehling's solution. It is precipitated by barium hydroxide as  $BaO(C_6H_{10}O_5)_3$ , and by basic lead acetate as  $PbO(C_6H_{10}O_5)_2$ .

When boiled with dilute nitric acid, glycogen yields oxalic acid. Boiled with dilute sulphuric or hydrochloric acid, it is converted into dextrose. It is not fermented by yeast, but diastase and saliva convert it into maltose and erythro-dextrin, a little dextrose being also formed. On the other hand, in the hydrolysis of glycogen in the liver, dextrose and not maltose is the chief product. (Compare p. 262.)

**Quantity of Glycogen in Flesh.**—During life the amount of glycogen in muscular tissue represents the balance between the quantity formed and that hydrolysed and is dependent upon a number of circumstances, among which are diet and work. The amount found varies with the kind of animal and the kind of tissue;

and since the hydrolysis of glycogen through the agency of enzymes goes on after death, it is also dependent on the age of the meat.

A. Bujard (*Forsch. Ber.*, 1897, 4, 47; abst. *Analyst*, 1897, 22, 160) has published the following determinations of glycogen made by the method of Niebel and Salkowski (*Z. Fleisch und Milkhvg.*, 1891, 185).

	Water %	Glycogen; direct %	Glycogen on dried substance %
Horse flesh.....	61.83	0.846	2.24
Horse flesh.....	72.90	0.174	0.64
Horse flesh.....	70.47	1.366	4.62
Horse flesh.....	71.84	0.59	2.09
Horse flesh smoked.....	43.00	0.108	0.19
Beef (ox).....	73.62	0.206	0.74
Beef.....	75.55	0.018	0.073
Veal.....	76.12	0.346	1.44
Veal.....	74.47	0.066	0.25
Pork.....	54.05	trace	trace
Pork.....	66.29		

Bujard also reports the following figures which were obtained by later methods. These methods, however, have now been superseded by Pflüger's. (See below and compare p. 265.)

	Water %	Glycogen direct		Glycogen in dried substance	
		Niebel method %	Mayr- hofer method %	Niebel %	Mayr- hofer %
Horse flesh.....	78.44	0.440	0.445	1.721	1.741
Horse flesh.....	74.87	0.600	0.520	2.388	2.069
Horse flesh.....	76.17	1.827	1.727	7.667	7.247
Horse flesh.....	76.00	0.592	0.610	2.466	2.542
Veal.....	74.6		0.086		0.342
Pork.....	75.6		0.186		0.744

W. Niebel (*Fleisch und Milch Hyg.*, 1891) gives the following figures showing the amounts of glycogen found in the flesh of various animals. The analyses were made by the older methods.

Kind	Age	Glycogen %
Horse meat. ....	3 hours	0.700
Horse meat. ....	3 hours	1.026
Horse meat. ....	1 day	0.373
Horse meat. ....	2 days	0.603
Horse meat. ....	3 days	0.523
Horse meat. ....	4 days	0.524
Horse meat. ....	5 days	1.072
Horse meat. ....	5 days	0.460
Beef. ....	4 hours	0.204
Beef. ....	1 day	0
Beef. ....	2 days	0
Beef. ....	1½ hour	trace
Beef. ....	5 days	0.076
Pork. ....	4 hours	0
Pork. ....	2 days	0
Mutton. ....	2 days	0

These figures show, in general, the decrease of glycogen in stored meat, and at the same time the varying amounts present in the same kind of meat and the irregularity of the decrease.

Niebel also reported the following figures showing the amounts of glycogen and dextrose in various kinds of meat of different ages.

Kind of meat	Age, days	On the original basis				On the dry and fat-free basis		
		Water %	Fat %	Dextrose %	Glycogen %	Glycogen %	Dextrose %	Total carbohydrates as sugar %
Horse. ....	8	75.2	3.5	0.417	0.812	3.810	1.957	6.190
Horse. ....		75.2	2.6	0.263	0.532	2.396	1.139	3.801
Horse. ....		75.3	2.6	0.142	0.744	3.397	0.648	4.421
Horse. ....		71.7	6.6	0.180	0.940	4.782	0.828	6.151
Horse. ....	8	71.9	7.1	0.222	0.606	2.886	1.057	4.387
Beef. ....		75.3	3.6	0.066	trace	trace	0.314	0.314
Beef. ....	1	75.3	3.6	0.190	trace	trace	0.900	0.900
Beef. ....		75.3	3.6	0.036	0.164	0.777	0.177	1.033
Beef. ....		75.3	3.6	0.071	0	0	0.336	0.336
Beef. ....		75.3	3.6	0.070	0	0	0.331	0.331
Beef. ....		75.3	3.6	0.210	0	0	0.336	0.336
Veal. ....	1	78.8	0.9	0.250	0	0	0.331	0.331
Veal. ....	1	78.8	0.9	0.250	0	0	0.331	0.331
Pork. ....		73.8	5.1	0.156	0	0	0.739	0.739
Pork. ....	2	73.8	5.1	0.100	0	0	0.479	0.479
Pork. ....		73.8	5.1	0.100	0	0	0.985	0.985
Mutton. ....		66.4	14.4	0.005	0	0	0.052	0.052
Mutton. ....		70.0	8.0	0.171	trace	trace	0.777	0.777

Niebel also gives figures for various sausages with and without horse flesh.

Because of the relatively large amount of glycogen in horse flesh, its determination has been used for the purpose of detecting horse meat in mixtures of meats with some success. W. Niebel considers the presence of more than 1% dextrose, after conversion of the other carbohydrates, on the basis of the fat-free meat, to be proof of the presence of horse flesh, in the absence of starch. However, because of the continued decomposition of glycogen after death, the failure to find more than this equivalent does not necessarily prove the absence of horse meat, and the very general practice of using sugar in the curing of meats introduces another uncertain factor.

Trowbridge and Francis (*J. Ind. Eng. Chem.*, 1910, 2, 215) obtained the following percentages of glycogen in beef liver and lean beef muscle:

Description of animal	Glycogen in liver %	Glycogen in lean muscle %
Slaughtered 2-3 hours after regular A.M. feed		
Very fat Short-horn, 4½ years	3.803	0.663
Lean Jersey cow, 6 years	2.337	0.697
Fairly fat Short-horn, 3½ years	1.210	0.243
Fat yearling Hereford	0.927	0.375
Fat Hereford, 20 months	0.820	0.309
Slaughtered 5-6 hours after regular A.M. feed		
Thin Hereford, 2 years	0.965	0.569
Very thin Hereford, 23 months	0.913	0.416
Thin Hereford, 11 months	0.625	0.158
Slaughtered 9 hours after regular A.M. feed		
Very fat Hereford, 3 years	1.160	.....
Fairly fat Hereford, 18 months	0.284	0.140

The above data show the greater tendency of older animals to store glycogen and the influence of the length of time elapsing after feeding before the animal is slaughtered on the amount of glycogen that remains stored in the organs and muscles. Furthermore, the glycogen-content of the muscle of the emaciated Hereford steer (0.416%) shows that, notwithstanding this emaciated condition and the fact that the entire carcass contained no fat which could be separated mechanically, the muscle still contained glycogen in considerable quantity.



Trowbridge and Francis also obtained (*loc. cit.*) the following percentages of glycogen in cow liver and horse flesh at various periods of time after slaughter.

Liver of cow	Glycogen %	Horse flesh	Glycogen %
After 2 hours, 13 minutes...	3.13	After 36 minutes.....	0.146
After 1 week .....	2.67	After 22 hours.....	0.072
After 15 days.....	2.34	After 3 days.....	0.013

These experiments show that the glycogen-content of beef muscle and beef liver ranges from 0.1 to 0.7, and from 0.2 to 3.8%, respectively; that starvation or extreme debility does not cause complete removal of glycogen from the muscle or liver; that the glycogen slowly decreases, but does not entirely disappear when the meat is kept at a temperature of 6.5° for over two weeks; that glycogen may be present even when the liver is unfit for food; that horse flesh is subject to an enzymic hydrolysis of the glycogen similar to that of beef; and finally that the glycogen content cannot be said to offer an absolute or even approximate basis for distinguishing beef from horse flesh.

Glycogen was first found in the liver, but has been more recently met with in many other parts of the body. It has been termed "Animal Starch," from its close analogy to soluble starch.

**Preparation of Glycogen.**—Glycogen may be prepared by rapidly cutting up the liver of an animal killed immediately previously, and throwing the fragments into five times their weight of boiling water. After boiling for a short time, the fragments of liver are mixed with sand and reduced to powder in a mortar, and then returned to the water, which is again boiled. The liquid is strained, and faintly acidified with acetic acid while still hot. The filtrate from the coagulated proteins is rapidly cooled, and the remaining proteins precipitated by the alternate addition of hydrochloric acid and potassio-mercuric iodide. The filtered liquid is mixed with such a volume of strong alcohol as to make it contain 60% of absolute alcohol, after which the precipitated glycogen is filtered off, washed first with 60% alcohol, and then with absolute alcohol and ether.

Kistiakoffsky (*J. Russ. Chem. Soc.*, **25**, 60; *abst. J. Chem. Soc.*, 1893, **62**, 618) prepares glycogen from the liver and muscles, taken

as before immediately after the death of the animal, by cold extraction. The material is rubbed up in an iron mortar, cooled to a very low temperature to prevent fermentation, and the homogeneous mass then extracted with ice-cold water containing 1 to 2% hydrochloric acid. This operation is repeated until the last extract ceases to give the glycogen reaction with iodine. If it is not essential that the whole of the glycogen should be extracted, water containing from 0.2 to 0.7% of acid may be used. The solution obtained is coloured by haemoglobin, and contains albuminous matters, which are precipitated by means of mercuric iodide. This precipitate is filtered off and washed with dilute mercuric iodide solution until free from glycogen. The glycogen is precipitated from the filtrate and washings by the addition of about one and a half volumes of alcohol. It is collected on a filter, and washed first with 75% alcohol, and then with ether-alcohol. After being dried over sulphuric acid, the product forms a white, amorphous powder, containing no nitrogenous compounds, and leaving only traces of ash on ignition. If dried in the air, a resinous mass difficult to powder is obtained. This method may be used for the estimation of glycogen in animal tissues, the results obtained being somewhat under those found by Brücke's method. (See further, Kistiakoffsky, *abst. J. Chem. Soc.*, 1896, **69**, 80.)

Brücke advocates the use of 0.1 to 0.3% solutions of alkali for the extraction of glycogen, instead of the 2% alkali solutions as sometimes used. If the extraction of the glycogen is effected with boiling water, the aqueous liquid contains, besides glycogen, alkali albuminates, gluten, and traces of peptone (Kistiakoffsky, *abst.*, *J. Chem. Soc.*, 1896, **69**, 80). These are all precipitated by hydrochloric acid and potassio-mercuric iodide, as already described.

Of the substances proposed for the extraction of glycogen, boiling water, trichloroacetic acid, sulpho-salicylic acid, and formaldehyde all extract albumoses, in some cases in considerable quantities, from the animal substances used. The separation of the albumoses is attended with some difficulty. Their presence may be conveniently recognised by treating the glycogen solution with a reagent containing 100 parts of sodium tungstate, 50 parts of phosphoric acid, 10 parts of concentrated hydrochloric acid, and 500 parts of water. On treatment with Millon's reagent, the dried precipitate

will show the presence of 0.02% of albumose. The presence of glycogen does not interfere. This test is due to D. Huizinga (*Pflüger's Arch.*, 61, 32; abst. *J. Chem. Soc.*, 1896, 68, 6), who finds that the best results are obtained when the liver or other animal substance is treated with a mixture of equal parts of a saturated solution of mercuric chloride and Esbach's reagent, made by dissolving 10 grm. of picric acid and 20 grm. of citric acid in water, and making the solution up to 1,000 c.c. This treatment does not extract the whole of the glycogen from the animal substance.

Pure glycogen is a snow-white, amorphous powder, readily soluble in water to form a solution which is usually, but not invariably, opalescent, and which becomes more limpid on adding acetic acid or an alkali. Glycogen is precipitated from its aqueous solution by alcohol whenever the alcohol amounts to 60% of the liquid. If the solution be quite free from salts, the separation is sometimes very difficult, but takes place instantly on adding a minute quantity of common salt. The precipitation of glycogen in liquids containing 60% of alcohol distinguishes it from the different varieties of dextrin, none of which is precipitated by alcohol of less than 85% strength. On the other hand, glycogen exactly simulates erythro-dextrin in its behaviour with a solution of iodine, which produces a port-wine colour, disappearing on heating, and returning as the liquid cools.

**Detection of Glycogen.**—Microscopically, glycogen can be detected in tissue preparations by means of Lugol's solution (iodine 1, potassium iodide 2, distilled water 200); but it should be noted that previous contact with water, and also contact with the water of the test solution, will dissolve the glycogen. Hence, tissue should be hardened in alcohol, not in aqueous solutions. The iodine stains the glycogen reddish-brown.

Chemically, use is made of the iodine reaction also for the detection of glycogen. Depending on the concentration of the glycogen solution in water, the colour produced by iodine varies from yellowish-brown, through reddish-brown, to deep red. Fifty to 100 grm. of the finely ground sample are boiled with an equal weight of water for 20 minutes, and the extract strained through fine cheesecloth free from starch. To the filtrate a few drops of iodine solution (iodine 2, potassium iodide 4, water 100) are added. The reddish-brown colour produced disappears on heating and reappears on cooling. (Compare p. 451.)

Pflüger makes use of 2 test-tubes of equal diameter, placing respectively an equal volume of water and the glycogen solution in them. Both are then heated to the same temperature for the same period, whereupon the colour disappears from the one containing glycogen and, on cooling, it reappears. In the presence of certain impurities the colour disappears slowly in the cold, rapidly on heating, and does not return. Evidently the iodine, under these conditions, enters into chemical combination with the impurity. To avoid this result, 10 c.c. of the impure solution are brought to a concentration of 3% potassium hydroxide, and 10% potassium iodide, and 50 c.c. of 96% alcohol added, and the liquid filtered. The filter is washed first with a mixture of 1 volume of 3% potassium hydroxide and 10% potassium iodide in water, and  $\frac{1}{2}$  volume of 96% alcohol, then with 60% alcohol, and finally with nearly absolute alcohol (97.8%). The precipitate is dissolved in water, the alcohol removed by evaporation on the water-bath, and the solution neutralised with acetic acid. Thus freed from the interfering impurity, the solution is used for the glycogen test previously described.

If starch is known to be present or is suspected, it can be precipitated by treatment with twice the volume of acetic acid (glacial), filtered off, and the test for glycogen applied to the filtrate.

**Estimation of Glycogen.**—Pflüger's (E. Pflüger, *Das Glycogen*, 1905; *Pflüger's Arch.*, 1906, **114**, 242) methods for the separation and estimation of glycogen have superseded the older ones, and these have now only a historic interest.

His methods will be followed here.

*Separation of the Glycogen.*—Fifty grm. of the finely hashed sample are treated in a flask with 50 c.c. of 60% potassium hydroxide and digested in a boiling water-bath for 3 hours with occasional thorough agitation. The solution is cooled and transferred to a beaker, 100 c.c. of water being added, a part of which is used to rinse the digestion flask thoroughly. The glycogen is precipitated with 2 volumes of 95% alcohol (200 c.c.). The precipitate is allowed to settle (not more than 24 hours) and the supernatant liquid is poured through a filter. The precipitated glycogen is now agitated three times successively with considerable 60% alcohol containing a few drops of saturated sodium chloride solution (equal to 7 mg. NaCl per litre), allowed to settle, and the wash-alcohol decanted through the filter. This treatment is followed by two treatments with

96% alcohol, one with absolute alcohol, three with ether, and one with absolute alcohol. The glycogen on the filter is washed with hot water into the beaker containing the rest of the glycogen, and the whole brought into solution by stirring with water.

*Determination of Glycogen.*—Three methods are available: (a) direct, (b) as dextrose after hydrolysis, (c) by means of polarisation.

(a) *Direct.*—Two mg. of sodium chloride per 100 c.c. water are added and the glycogen is reprecipitated with 2 volumes of 95% alcohol. The precipitate is brought on a filter, washed with 95% alcohol containing 7 mg. sodium chloride per litre, then with absolute alcohol, and finally with ether. It is dried to constant weight.

(b) *By Hydrolysis and Estimation as Dextrose.*—The glycogen solution is neutralised with hydrochloric acid of sp. gr. 1.19, drop by drop, and washed with hot water into a 500 c.c. flask, 25 c.c. of hydrochloric acid of sp. gr. 1.19 are added, and the volume made up nearly to the mark, leaving sufficient space for neutralising after the inversion. The solution will contain approximately 2.2% hydrochloric acid. Hydrolysis is accomplished by digestion in a boiling water-bath for 3 hours, after which the solution is made slightly alkaline by adding 60% potassium hydroxide solution, drop by drop, from a burette. The volume is made up to the mark, and reducing sugar (dextrose) determined by Allihn's method or, better, Pflüger's improvement of this method (Abderhalden, *Biochemischen Arbeitsmethoden*, 2, 174). (See Vol. I.)

To obtain the amount of glycogen, the dextrose result by the gravimetric method is multiplied by 0.927.

(c) *By Polarisation.*—The faintly alkaline glycogen solution is made weakly acid, and, disregarding the slight precipitate which forms, is washed into a volumetric flask of 100 or 200 c.c. capacity. An aliquot portion is filtered and used for the polarisation. The specific rotation for glycogen is 196.6°. It is best to take the mean of several readings and also to take readings at  $\frac{1}{2}$  and  $\frac{1}{4}$  the original concentration. Pflüger believes the rotation depends not only upon the concentration but also upon the size of the colloidal particles.

Starkenstein (*Biochem. Z.*, 1910, 27, 53) calls attention to a source of error in Pflüger's method. Ferric hydroxide, which may be present, is capable of absorbing large quantities of glycogen. The insoluble portion obtained after treating the tissue with potassium hydroxide solution should be dissolved in hydrochloric acid

and the glycogen estimated in the solution. Unless this is done, the error may amount to as much as 50% of the total glycogen present.

Bierry and Gruzewska (*Compt. rend.*, 1913, **156**, 1491) suggest the following method, which they claim gives excellent results:

Place 25 grm. of finely hashed meat in a flask containing 25 c.c. of potassium hydroxide solution. Heat for 15 minutes till the tissue is completely dissolved, then in an autoclave at 100° for 30 minutes. Cool, neutralise to litmus with hydrochloric acid, make up to 100 c.c., and heat in an autoclave at 120° for 30 minutes. Cool and neutralise with sodium hydroxide. Precipitate the proteins with mercuric nitrate. Filter the liquid brought up to 300 c.c. with the wash water and add zinc powder to remove excess of mercury. After several hours a colorless liquid is obtained. Estimate the dextrose in a 10 c.c. aliquot portion, and multiply by 0.927 to obtain the weight of glycogen.

Burghard and Paffrath (*Z. Kinderheilk.*, 1927, **45**, 68) recommend that, on account of the post-mortem decomposition of glycogen in muscle and liver, the total carbohydrate content should be determined in liver and the liver-glycogen calculated from this, the constant difference being 0.23.

Soz (*Boll. Soc. ital. Biol. sper.*, 1929, **4**, 1155) gives a method for determining glycogen in adipose tissue: Rapidly weigh 0.1–0.2 gram of adipose tissue and place in an extraction thimble. Extract with boiling alcohol for 7–10 hours. Macerate the residue with 1–2 c.c. of 60% potassium hydroxide on the water-bath for 1 hour, and then transfer to a centrifuge tube with 50 c.c. of 96% alcohol and 1–2 drops of concentrated sulphuric acid. After a few hours centrifuge for 10 minutes at 3000 r. p. m. Dissolve the precipitate in water, reprecipitate with alcohol, centrifuge again, redissolve in water, and add hydrochloric acid to give a concentration of 2.2%. Boil the solution for 3 hours and determine the glucose. The identification of glucose is confirmed by fermentation and by osazone formation.

The methods of estimating glycogen, lower carbohydrates, and lactic acid have been critically examined by Boyland (*Biochem. J.*, 1928, **22**, 236). He presents an improved method for the determination of lactic acid, which involves steam distillation combined with oxidation.

**Dextrin and Sugars.**—These are hydrolytic products of glycogen. They do not remain long in the tissues but soon enter into metabolic processes, the sugar (dextrose) being destroyed by a glycolytic enzyme.

*Estimation of Reducing Sugar (Dextrose).*—One hundred grm. of the finely hashed meat are extracted successively with 200, 100, and 100 c.c. of water by boiling in an evaporating dish. Each time the solution is poured through fine linen or cheese cloth and the meat wrung out by hand in the same medium (suitable precautions being taken) into a 500 c.c. flask. The solution is clarified with lead sub-acetate, the lead removed by sodium sulphate in an aliquot portion, and the reducing sugar determined as dextrose by Allihn's or other standard method.

**Lactic Acid.**—Lactic acid (hydroxypropionic acid, ethylidene lactic acid)  $\text{CH}_3\cdot\text{CHOH}\cdot\text{COOH}$ , occurs in 3 forms of biological interest, the dextro-, lævo-, and inactive ethylidene lactic acids. However, only the dextro modification (para- or sarco-lactic acid) appears to be present in animal tissues and secretions. The total quantity in flesh varies from 0.1 to 1.0%.

*Estimation of Lactic Acid.*—Meissner (*Biochem. Z.*, 1915, **68**, 175) concentrates the lactate solution to a syrup, then treats it with 5 c.c. of phosphoric acid and rubs with dry calcium sulphate to form a powder. This is extracted with ether for 7–9 hours, thus removing the lactic acid quantitatively. The extract is concentrated, and the acid titrated, or estimated as carbon monoxide by conversion into the barium salt, concentrating to dryness, and decomposing with sulphuric acid in an atmosphere of carbon dioxide. The decomposition is usually complete at  $100^\circ$ , but the flask may be heated to the boiling point of sulphuric acid.

Yoshikawa (*Z. physiol. Chem.*, 1913, **87**, 382) estimates *d*-lactic acid by measuring the rotation of its lithium salt.

**Inositol.**—Inositol,  $\text{C}_6\text{H}_{12}\text{O}_6$  or  $\text{C}_6\text{H}_6(\text{OH})_6$ , is an aromatic compound formerly classified with the hexoses and found in many plant tissues, as well as in animal tissue. It is the optically inactive modification which is found in the latter. The dextro and lævo modifications are known. Inositol is non-fermentable and does not reduce Fehling's solution, although it changes its colour. Horse flesh contains 0.003% inosite, according to Jacobsen.

*Detection of Inositol.*—All of the inositols upon oxidation with nitric acid yield coloured hydroxyquinone derivatives. In carrying

out this test Scherer's method (*Liebig's Ann.*, 1850, **73**, 322; 1852, **81**, 375) is generally used: Treat a small amount of the material to be tested with a little nitric acid and evaporate on a water-bath almost to dryness. Add a little ammoniacal barium chloride or calcium chloride, and again evaporate the solution. If inositol is present, a beautiful rose-red colour will develop. As little as 0.5 mg. of inositol may be detected.

Seidl (*Chem. Ztg.*, 1887, **11**, 676) has modified the test by using ammoniacal strontium acetate to develop the colour, and in this way 0.3 mg. may be detected.

Salkowski (*Z. physiol. Chem.*, 1910, **69**, 478) has still further modified the Scherer test as follows: Dissolve a small amount of the substance in 1-2 drops of nitric acid, add a few drops of 10% calcium chloride solution, then a few drops of 1-2% platinic chloride solution, and evaporate the whole in a porcelain dish. The presence of inositol is indicated by a rose-red to brick-red colour, the test being sensitive to 0.1 mg.

**Nitrogenous Extractives.**—E. Fischer (*Ber.*, 1897, **40**, 549, 559, 1839, 2226, 2604, 3089) classified many of the nitrogenous extractives of meat under the term "purine bases" considering them as derivatives of purine  $C_5N_4H_4$ . The relationships will not be considered in detail here, but can be ascertained by reference to the literature.

They are formed not only as products of protein metabolism, but some of them also as hydrolytic products of proteins (especially nucleins) and as the result of bacterial decomposition of the proteins. At one time some of the purine bases were confused with the more active poisonous products of bacteria (the toxins), and the terms "ptomaines" and "leucomaines" were introduced, the former referring to poisonous basic substances produced by bacteria and the latter to basic substances produced by metabolism in the animal body. The word ptomaine has persisted and is still currently used, especially in the expression "ptomaine poisoning" which is popularly applied by physicians and laymen to various sorts of sickness resulting from eating spoiled animal and vegetable foods.

Caffeine, theobromine, and other bases derived from plants are closely related to the meat extractives of the purine group.

Wilson (*Chemistry of the Nitrogenous Extractives of Muscle Tissues*, Thesis, Yale University, 1914) has reviewed in detail the work of



recent years on the nitrogenous extractives of muscle. The following is a summary of the various nitrogenous compounds thus far found in the muscular tissue of both vertebrate and invertebrate animals:

*Alanine*.—In beef extracts and the muscle of lobster, salmon, tunny, snapper, crab, and dried codfish.

*Glycocoll*.—In scallop, mussel, crab extract, and shell fish; none in octopus.

*Glutamic Acid*.—In beef extract and dried codfish.

*Leucine*.—In crustacea, cephalopods, spiders, and insects; specifically in crab extract, lobster, cuttlefish, sardines and crab.

*Tyrosine*.—In crab extract, lobster, sardines, snapper, crab, and many invertebrates.

*Histidine*.—In beef extract, bonito, tunny, salmon, and sardines.

*Arginine*.—In crab extract, lobster, crab, and clam.

*Lysine*.—In crab extract and lobster.

*Proline*.—In lobster.

*Tryptophane*.—In crab.

*Taurine*.—In oyster, cuttlefish, octopus, frog, alligator, cephalopods, mollusca, periwinkle, abalone, beef extract, and dried codfish.

*Anhydride of d-alanyl-d-alanine*.—In beef extract.

*Carnosine (Ignotine)*.—In beef extract, muscles of ox, bonito, tunny, salmon, eel, calf, rabbit, sardine, horse, and pig. None found in liver or kidney extracts. Several investigators have failed to obtain it from invertebrate muscle.

*Methylguanidine*.—In beef extract and muscle of ox, haddock, calf, horse, and codfish. It has been found in liver extract but not in kidney extract.

*Creatine and Creatinine*.—In the muscle of practically all vertebrates.

*Trimethylamine*.—In fish and invertebrate extracts. It is possibly present only in small amounts in living muscle.

*Choline*.—In beef extract, crab extract, dogfish, and in many plant and animal tissues.

*Neurine*.—In beef extract and haddock.

*Carnomuscarine*.—In beef extract.

*Betaine*.—In sugar beet and many plant and animal extracts; also in muscle extracts of shrimp, crab, oyster, clam, dogfish, cuttlefish, octopus, mussel, dried codfish, scallop, periwinkle, and lamprey.

*Neosine*.—In beef extract, crab extract, and ox muscle.

*Carniline*.—In beef extract and muscle or ox, calf, horse, and pig; not in kidney or liver extracts.

*Myokynine*.—In dog and horse.

*Obliline*.—In beef extract.

*Hypoxanthine, Xanthine, Guanine, and Uric Acid*.—In muscle extracts.

*Carnine*.—In beef extract and horse muscle.

*Inosinic Acid*.—In beef extract.

*Methylamine*.—In flesh of coot.

*Iminazoethylamine*.—In tunny.

*Urea*.—Probably present in small amount in all muscle extracts. Unusually large amounts in muscle extracts of cartilaginous fishes.

*Vitiline and Creatosine*.—In beef extract.

*Canirine*.—In crabs and snapper.

*Crangiline and Crangonine*.—In crab extract.

*Melolonthine*.—In cockchafer.

Wilson gives in his thesis an extensive bibliography of muscle extractives. The reader is also referred to the papers by Ackermann, Becker, Blaha, Bottazzi, Buglia and Constantino, Cabella, Demjanowski, Dietrich, Einbeck, v. Fürth, Gulewitsch, Jansen, Jona, Krimberg and Israilsky, Mauthner, Mendel, Micko, Myers and Fine, Smorodinzew, Suzuki, Wilson and Yoshimura, which have appeared during the years 1913-16, chiefly in *Z. physiol. Chem.*, *J. Biol. Chem.*, *Biochem. Z.*, and *Amer. J. Physiol.* (see also Vol. VIII.

#### **Animal Bases).**

*Creatine*, methylglycocyamine, methylguanidine acetic acid,

$C_4H_9O_2N_3$ ,  $NH:C \begin{matrix} \nearrow NH_2 \\ \searrow N(CH_3)CH_2COOH \end{matrix}$  is constantly present in

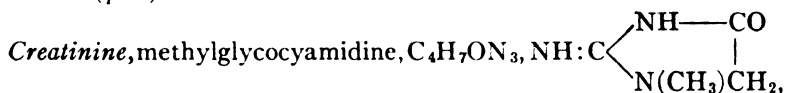
flesh, in which it is found to the extent of 0.1-0.35% and, with creatinine, is an especially characteristic constituent of meat extracts (*q. v.*). It was discovered by Chevreul in 1834 in meat extract and was investigated by Liebig in 1847. It crystallises with 1 molecule of water in colourless rhombic prisms, m. p. 100°, with loss of water. It has a neutral reaction in water, in which it dissolves readily when the water is heated, and a slightly bitter flavour. It dissolves in alcohol less easily than in water, and in ether is nearly insoluble.

When its aqueous solution is acidified and digested, creatine is completely converted into its anhydride creatinine. With barium hydroxide solution it takes up the elements of water, forming urea and sarcosine.

The amounts of creatine reported in flesh of various kinds are as follows (König, *Nahrungs und Genussmittel*, 2, 422): horse, 0.07–0.22%; rabbits, 0.21–0.34%; swine, 0.12%; cattle, 0.19–0.28%; duck, 0.20%; chickens, 0.21–0.33%.

Creatine can be prepared from muscular tissue (or, better, from meat extract) by extracting with boiling water, filtering, clarifying with lead acetate, filtering, removing the excess of lead with hydrogen sulphide, filtering, evaporating at low temperature, and purifying the crystals which separate by recrystallising.

Methods for the estimation of creatine are given under water extracts (*q. v.*).



is invariably present in flesh along with creatine. It is, therefore, present in beef extract and in larger proportion than creatine. It also occurs constantly in urine (0.25%). It can be prepared from creatine by digestion of the aqueous solution with acids. Creatinine crystallises in colourless rhombic prisms and dissolves easily in cold water and alcohol. It is a much stronger base than creatine, can expel ammonia from ammonium salts, and forms crystalline salts with acids. Bases cause creatinine to take up water with formation of creatine. Boiled with barium hydroxide it is decomposed into methylhydantoin and ammonia. Sodium phosphomolybdate precipitates it from acid solution. Zinc chloride forms a characteristic compound with it which is precipitated from solutions as a crystalline powder scarcely soluble in water and insoluble in alcohol. It is also precipitated by picric acid from concentrated solutions, and with the same reagent in alkaline solutions gives a reddish-yellow colour which is made the basis of Folin's method for its estimation. (See pp. 272, 299, 301 and under **Meat Extracts**, pp. 497, 507.)

A method for the estimation of creatinine is given under water extracts.

*Xanthine*, 2:6-Dioxypurine,  $C_5H_4O_2N_4$ , is widely distributed in animals and plants, being found in muscle, liver, spleen, pancreas,

and in potatoes, beets, and tea. It was discovered by Marcet in urinary calculi in 1823. As prepared from flesh, it is white and amorphous or forms granular masses of crystalline leaves, or, with 1 molecule of water, it crystallises in rhombic plates. It is insoluble in alcohol and ether, but dissolves in about 14,000 parts of water at 16° and 1,400 at 100°. It is but slightly soluble in dilute acids, easily in alkalies. It is decomposed at 156°, with formation of ammonium cyanide, carbon dioxide, formic acid, and glycoll. With nascent hydrogen it forms sarcine (hypoxanthine). It can be synthesised by heating hydrocyanic acid with water and an excess of acetic acid in a sealed tube to 145°. Its hydrochloride crystallises in needles and hexagonal prisms. The platini-chloride compound crystallises in yellow prisms.

When xanthine is evaporated to dryness with nitric acid in a porcelain dish on the water-bath, a yellow residue is left, which when treated with sodium or potassium hydroxide becomes first red and then purple. This is known as the *murexide test*. *Weidel's reaction* is applied by treating a xanthine solution in a test-tube with chlorine water (or with hydrochloric acid and potassium chlorate), heating, and evaporating carefully to dryness in a porcelain dish. If the dish and contents are placed in contact with ammonia fumes (as under a bell-jar) first a red, then a purple colour are developed.

The amount of xanthine in muscular tissue is small. According to Kossel, the flesh of pigeons and hens contains from 0.01% to 0.1%.

*Hypoxanthine*, *Sarcine*, 6-Oxypurine,  $C_5H_4ON_4$ , is constantly present in muscle and various glands, accompanying xanthine. It also occurs in plants. Sarcine forms white crystalline needles, which dissolve with difficulty in cold, but easily in hot water (70–80 parts). It is nearly insoluble in alcohol, soluble in weak alkalies and ammonia and in acids. It does not give the murexide test.

The following amounts are reported by Hofmann in the flesh of animals (*Lehrbuch der Zoochemie*, 1879, 83): cattle, 0.016–0.022%; horse, 0.013–0.014%; rabbit, 0.026%; dog, 0.025%.

*Uric acid*,  $C_5H_4O_3N_4$ , occurs in muscle, blood, urine (especially in carnivorous animals, hippuric acid being characteristic of herbivorous), and in the excrements of birds, reptiles, and insects. It was discovered by Scheele in 1776 in urinary calculi. It forms a white, crystalline, granular powder, is odourless and tasteless, insoluble in alcohol and ether, and difficulty soluble in water. (In 15,000 parts

at  $20^{\circ}$ , 1,800 parts at  $100^{\circ}$ .) On evaporating to dryness with nitric acid a yellow residue is left which turns purple-red if moistened with ammonia, and violet with sodium or potassium hydroxide (*murexide test*). Uric acid is a weak dibasic acid. Uric acid and urea form the chief nitrogenous end-products of protein metabolism and are of immense importance to the physiologist and physiological chemist.

*Urea, carbamide*,  $\text{CO}(\text{NH}_2)_2$ , the most important nitrogenous end-product of protein metabolism, occurs in the urine of mammals and especially in that of carnivorous animals. In the urine of man it is present in the proportion 2–3%, and a full-grown man excretes about 30 grm. daily. Urea occurs also in all animal fluids and tissues, including muscular tissue. It was discovered by v. Rouelle in 1773, in urine, and was synthesised by Wöhler in 1828 from ammonium isocyanate—a fundamental work in the history of organic chemistry. It can be prepared from urine by evaporation and treatment of the concentrated urine with strong nitric acid. The precipitate is dissolved in boiling water, decomposed with barium carbonate, the mixture evaporated to dryness, and the urea dissolved out with alcohol.

Urea crystallises in colourless rhombic prisms or needles, which taste somewhat like saltpetre. It is readily soluble in water (1 part), in alcohol (15 parts), and is practically insoluble in ether. It melts at  $132^{\circ}$  and above this temperature decomposes. When heated above  $100^{\circ}$  with water, or when boiled with acids or alkalis, it decomposes into carbon dioxide and ammonia. The same reaction is brought about by certain bacteria.

*Carnine*,  $\text{C}_5\text{H}_2(\text{NH}_3)_2\text{O}_3\text{N}_4$ , was discovered by Weidel in 1871 in meat-extract. It occurs also in the muscle of frogs and flesh of fish. It is insoluble in alcohol and ether, difficulty soluble in cold, easily soluble in hot, water. It gives *Weidel's reaction*.

*Inosinic acid*,  $\text{C}_{10}\text{H}_{13}\text{O}_8\text{N}_4\text{P}$ , occurs in small quantity in the muscles of rabbits, birds, (turkey 0.21%) and fish.

*Taurine*, aminoethylsulphonic acid,  $\text{SO}_2 \begin{cases} \text{C}_2\text{H}_4\text{NH}_2, \\ \text{OH} \end{cases}$  occurs in

very small quantities in the muscular tissue of mammals and in molluscs, also in taurocholic acid in the bile of cattle. It was discovered by Gmelin in 1824. It crystallises in monoclinic prisms which are soluble in hot water, insoluble in alcohol.

**Carnosine.**—This extractive appears to be assuming more importance, since several investigators have reported methods of determining it. The best method seems to be that of Hunter (*Biochem. J.*, 1921, **15**, 689; 1922, **16**, 640) who extracts the tissue with water, precipitates the proteins, and determines the carnosine by diazotising by a modification of the method of Koessler and Hanke (*J. Biol. Chem.*, 1919, **39**, 497). Hunter criticises the method of Clifford and Mottram and calls attention to errors due largely to impure carnosine. To this Clifford and Mottram have replied (*Biochem. J.*, 1928, **22**, 1246).

**Amino Acids.**—These are found in various flesh foods, animal fluids and tissues, and also occur as hydrolytic products of the proteins when these are digested with mineral acids, and as products of bacterial decompositions. The following belong to this class: Glycocoll, amino-acetic acid  $\text{NH}_2\text{CH}_2\text{COOH}$ ; sarcosine, methylglycocoll,  $\text{CH}_3(\text{NH}\cdot\text{CH}_2)\text{COOH}$ ; leucine,  $\alpha$ -amino-isobutyl acetic acid,  $\text{C}_5\text{H}_{10}\text{NH}_2\text{COOH}$ ; tyrosine, *p*-hydroxyphenyl- $\alpha$ -aminopropionic acid,  $\text{C}_6\text{H}_4(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ .

### Enzymes of Meat

Various enzymes have been identified in meat, not so much by methods of isolation but principally by the reactions of muscular tissue under definite conditions *in vitro*, or by inference from reactions known to occur in the living tissue. Only the more important ones are considered here, since many have been reported on insufficient authority or evidence. Of them all, the most important from the practical and analytical standpoint is the proteoclastic enzyme, and next to this the fat-splitting enzyme.

**Proteoclastic Enzyme—Protase.**—Evidence of the presence of this enzyme is afforded by the fact that after the onset of *rigor mortis*, which results in a stiffening of all the muscular tissues, when meat is held, as is customary, in the chill-room or cooler at temperatures varying usually from 1 to 3° (34 to 38° F.), a progressive softening of the muscular tissue occurs, which makes it more tender and hence more palatable. Practical use is made of this enzyme by packing houses and markets in the ordinary method of holding meats, as outlined above, to ripen them. This ripening is entirely distinct from bacterial action and, as commonly practised, is not accompanied by the latter. It is more fully described elsewhere.

The enzyme can be prepared in an impure condition by extracting fresh, finely ground, lean beef several times with chloroform water, saturating with sufficient alcohol to make a 60% solution, shaking, allowing the mixture to stand overnight, and decanting the supernatant liquid from the precipitate. The latter is dissolved in water and precipitated with sodium phosphate and calcium chloride in molecular proportions. After separating the liquid, the residue is taken up with chloroform water and filtered, the filtrate dialysed, and precipitated with alcohol (to 80%). The precipitate is allowed to settle, filtered off, washed with 95% alcohol, and dried at a low temperature.

Thus prepared, the enzyme appears to be most active in a weak alkaline solution (such as occurs in living muscle); but in the case of meat (after *rigor mortis*) it is obvious that it acts in an acid medium.

**Lipoclastic Enzyme—Lipase.**—This enzyme is associated with all the fatty tissues of the animal body, and it can be demonstrated in them by the simple process of holding the finely ground tissue (best emulsified with an equal weight of water) under aseptic conditions at a temperature of 40°, and observing the increase in fatty acids. Whether it is intimately associated with muscular tissue, free from fat, is questionable; but connective tissue containing fat is so closely bound up with muscular tissue, that it would be practically impossible to prepare muscular tissue which would contain no fat-splitting enzyme. The lipase of animal fats appears to be active not only at the optimum temperature (near 40°) but also at much lower temperatures, even below the freezing-point. But as the temperature is reduced below 40° the activity is progressively lessened, until just above the freezing-point it is slight indeed, and at -10° the formation of fatty acids cannot be detected in ordinary animal fats, even after the lapse of two years. However, if finely ground pancreas, which contains much fat-splitting enzyme, is mixed with ground-up fat and the mixture emulsified, an increase of fatty acid can be demonstrated at -10°. The activity of lipase at low temperatures may depend on its insolubility in water. These points are of importance to the food analyst, as is indicated in another place.

The more quickly fatty tissue is rendered after death, and the lower the temperature used in rendering, the less will be the amount of fatty acids, found by analysis, in the rendered fat. In carefully rendered animal fats the percentage of free acid (as oleic) is usually

from 0.2 to 0.35%. It is assumed, therefore, that fats as they occur in the body during life are neutral or practically so, that is, they contain no appreciable amount of fatty acid. This would indicate that lipase is formed in fatty tissue upon the death of animals, or that during life there is some counterbalancing tendency to offset the formation of fatty acids. It should be noted that it has been shown that some fat-splitting catalytic agents (Twitchell reagent, lipase) are also synthesising agents under appropriate conditions (Dunlap and Gilbert, *J. Amer. Chem. Soc.*, 1911, **33**, 1787). Fat splitting occurs in the presence of considerable water with low glycerol concentration, fat synthesis with high glycerol concentration, fatty acid being present (Twitchell, *J. Amer. Chem. Soc.*, 1907, **29**, 566). Dietz (*Z. physiol. Chem.*, 1907, **52**, 279) showed the synthetic action of lipase in a mixture of a little butyric acid and much *iso*-amyl alcohol. The amount of fatty acid found in the fats associated with meats may be as much as 10% (as oleic acid), for example, in old cured hams and summer sausage.

**Amylolytic Enzyme—Diastase.**—Diastatic enzymes occur in the saliva, pancreatic juice, blood, lymph, and liver, and to a less extent in most tissues. Dextrin, maltose, and dextrose have been demonstrated in muscular tissue.

**Maltase, oxydase, catalase, a glycolytic enzyme, and myosin enzyme** have all been claimed to occur in muscular tissue. The last, if it exists, is not identical with fibrin enzyme (Halliburton).

**Nitrate-reducing enzyme** has been claimed to exist in muscular tissue by several observers. Richardson has failed to demonstrate its presence in fresh meat, although nitrate reduction occurs in meat as soon as bacteria begin to grow therein, and in this fact is probably to be found the reason for the reports of its presence. There are excellent reasons, also, in connection with the curing of meats, when saltpetre is used, which indicate that such an enzyme (or reducing substance) does not exist in fresh meat. If it were generally present, all saltpetre-cured meats would invariably be red, which is not the case.

## GENERAL METHODS OF SAMPLING AND ANALYSING MEATS

### Methods of Sampling

As a rule, it is better to separate the sample of meat first into its parts, such as lean, fat, gristle, and bone, and to analyse any or all



of these separately. In ascertaining the composition of the whole animals or of the larger cuts, these separated portions are weighed, in order to afford a basis for the final calculation. Ordinarily it is only the lean and fat and especially the lean with its small proportion of inseparable fat tissue which is analysed. In the great majority of cases only the last is analysed. Large and small butcher knives and a bone saw are required for the dissection. If the sample is in the form of a medium or large-sized piece, a convenient procedure is first to subdivide it into parallel slices about 0.5 to 1 in. thick, later removing from these the fat, gristle, and bone, and finally cutting the lean into small cubes. These cubes are then run preferably through an "Enterprise" hasher once or twice and the ground sample thoroughly mixed. In lieu of the "Enterprise" hasher an ordinary household chopping bowl and knife may be used to advantage.

In handling samples of the meat products their perishability should always be kept in mind and every effort made to prevent decomposition. Low temperatures afford the best means of preservation and, if the sample is to be kept for any length of time, the small cubes as obtained by the method described should be placed in air-tight jars—such as Mason jars—and held in frozen condition. If juice separates during thawing, it should be well incorporated with the sample after hashing. Antiseptics should be used only in cases where low temperatures are not available for preservation and are never to be recommended. Special methods for sectioning and sampling cured meats are given under this heading (page 408).

Some additional suggestions and precautions are given by Moulton, Trowbridge, and Haigh (*Missouri Agr. Expt. Sta., Research Bull.* 55, 1922) in a report of the methods used by them in preparing parts of the beef animals for analysis. The following is quoted from their work.

"The samples of the soft tissues and parts were passed through a power grinder (Enterprise) equipped with four sets of plates, each plate having holes of a different size than the other. Samples were ground through the coarser plate and then through the next size. The samples were well mixed and quartered down, if necessary, and then ground through a finer plate. The large samples were then quartered again and ground through the finest plate. Very homogeneous and fine samples were easily obtained in this manner. An especially difficult sample to make uniform was the respiratory

system. The cartilaginous rings of the trachea would partly remain behind in the mill, while the softer lungs were squeezed out past them. By means of a knife these rings were finely cut and mixed with the lungs. The hide sample was cut into thin strips with a knife, alternate strips being rejected in the larger samples. The strips were then cut into short lengths and ground through the mill already described. The grinding of the sample proceeded very slowly, but with repeated grindings the work advanced more rapidly and a final uniform and fine sample was obtained.

"The work of preparing and grinding the samples proceeded as rapidly as possible until the samples were in a position where there was no danger of decomposition or change. The samples were kept in jars provided with rubber gaskets, glass tops, and metal clamps so that no loss of moisture could occur. They were kept in cold storage at a temperature just above freezing, so that they remained fresh for analysis.

"The skeleton samples were ground through a Mann green bone grinder, mixed well and sampled. From this smaller samples were weighed out directly and rapidly, in triplicate, in tared porcelain evaporating dishes. The size of the samples varied according to the coarseness or fineness of the bone. For finely ground samples 25 to 40 grm. were considered sufficient while for coarse samples 100 grm. or even more were sometimes taken. The dishes containing the weighed samples were at once placed in vacuum desiccators and dried to a constant weight within 25 or 30 mg. They were then extracted with ether in specially constructed Soxhlet extractors. The residue was saved, the triplicates combined, and the whole ground in a steel mill until fine enough to pass through a millimeter sieve. The sample was allowed to become air dry and saved for a complete analysis later.

"Samples of horn and hoof were dried and reduced to a fairly fine state with a horseshoer's rasp. A drug mill was then used to reduce the material to a finer state."

The Association of Official Agricultural Chemists prescribes (*Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, published by the Association, Washington, 1925, 237) the following methods for the preparation of samples.

"Because of the high water content of meats, care must be taken to prevent loss of water during preparation and subsequent handling of sample. For this reason small samples are undesirable and the ground material should be kept in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in the following manner:

"(a) *Fresh Meats, Dried Meats, Cured Meats, Smoked Meats, Etc.*—Separate as completely as possible from any bone and pass rapidly through a food chopper three times, thoroughly mixing after each grinding. Begin all determinations as soon as practicable after grinding and mixing. In the case of delay before the examination is started, chill the sample to inhibit decomposition.

"(b) *Canned Meats.*—Pass the entire contents of a can through a food chopper as directed under (a).

"(c) *Sausages.*—Remove from casings and pass through a food chopper as directed under (a).

"Dry the portions of the samples under (a), (b), and (c) not needed for immediate analysis, either *in vacuo* below 60° C. or by evaporating on a steam bath two or three times with alcohol. Extract the fat from the dried product with gasoline (b. p. below 60°) and allow the gasoline to evaporate spontaneously, finally expelling the last traces by heating for a short time on a steam bath. Do not heat the sample or the separated fat longer than necessary because of tendency to decompose . . . "

The fat may be reserved for any examination desired by keeping it in a cool place and completing the examination before it becomes rancid.

### Methods of Analysis

**Moisture.**—From 5 to 20 grm. of the finely hashed sample are weighed—best by difference from a weighing bottle—into a watch glass or metal moisture dish and the moisture determined by difference after drying under low pressure in a vacuum drying oven heated by electricity. The exact temperature and pressure can be varied according to circumstances, but a pressure of 60 mm. or less and a temperature of 40–50° are desirable. The sample should be taken to constant weight, but a few trials at a given pressure and temperature will suffice to set a minimum limit at which desiccation is complete.

Where a vacuum oven is not available, moisture may be determined by difference after drying in one of the forms of hydrogen ovens in a current of hydrogen.

For ordinary work it is sufficiently accurate to dry the sample in the air-bath (oven) at the temperature of boiling water or better at 100–105°. The bulb of the thermometer should be located near the drying sample.

The A. O. A. C. methods prescribe for the vacuum oven method a temperature of boiling water and a pressure not to exceed 100 mm of mercury.

During the heat drying of substances such as meat which contain fat fairly readily oxidised, it has been the experience of the author that, as the heating and drying proceeds, the weight of the sample reaches a minimum. After this there is a short period of gain in weight followed again by further losses. These changes are the result of two or more processes: first, a loss of moisture; second, an oxidation of the fat at low water content with a gain in weight; third, further oxidation resulting in slight losses of organic matter. Water losses may continue during the second and third processes. The methods given above attempt to meet this situation. For rapid and not too meticulous work the methods are suitable, but for careful work one must employ drying without heat.

*Desiccator Methods.* Richardson (**Meat and Meat Products**, Allen's *Commercial Organic Analysis*, 4th Ed. Vol. VIII, p. 296) has suggested that moisture may also be estimated by drying 5–10 grm. of the sample in the vacuum desiccator in the presence of sulphuric acid (J. König, *Nahrungs und Genussmittel* 3, 25, Berlin, 1910). This method is slower than that of the vacuum oven, and it is important that the sample be spread over the bottom of the dish in a thin layer, in order to prevent decomposition during drying. The Hempel form of vacuum desiccator is best for the purpose.

As a result of his experience with the analysis of beef animals at Missouri, the present author recommends the use of Schleicher and Schüll extraction shells, or, better still, glass tubes of the same size with a constricted bottom opening on which a disc of wire gauze is placed. The shells or tubes are filled with fat-free absorbent cotton lightly packed into the tube. These shells or tubes are extracted with ether, dried, and weighed to constant weight, employing counterpoised glass stoppered weighing bottles. The use of such

weighing bottles is very convenient, since the weight of the tube and contents is given directly and no complications arise from a broken weighing bottle. Scheibler vacuum desiccators, six inches in diameter with stopcocks in the lid, are filled to a depth of an inch with C. P. sulphuric acid (sp. gr. 1.84). A brass gauze or porcelain plate is placed on the shelf of the desiccator, and one-half inch above this, supported by corks or rubber stoppers, a second gauze covered with clean filter paper is placed. It is necessary to have the ground glass surfaces and stopcocks fit well. A lubricant of three parts of hard paraffin wax and five parts of yellow vaseline is prepared by melting together these ingredients and allowing the mixture to cool slowly. In cool weather more vaseline is used, and in hot weather a little more paraffin wax, to give the mixture the proper consistency. A lubricant containing rubber commonly used for stopcocks can be added in small quantities to a somewhat thinner mixture of paraffin and vaseline, thereby giving very desirable properties to the mixture.

The thoroughly mixed sample is placed in a weighing bottle provided with a short aluminium spoon or scoop. These can be made as needed out of sheet aluminum of the proper thickness. Samples, preferably in triplicate, of 3 to 5 grm., are weighed out on to the flattened cottonpad made from the absorbent cotton in the tube or shell. A small plug of cotton is left in the bottom of the tube. It is convenient to spread part of the cotton out on a porcelain plate, place the meat upon it in a thin layer, and then roll the cotton and meat up into a cylinder which is replaced in the extraction tube. Part of the cotton removed and not used is employed to clean any particles of meat or fat which may have touched the porcelain plate. A little experience renders one quite adept at the feat and no loss of substance occurs. By this means the meat is separated into a sufficiently thin layer so as to allow thorough drying and extraction.

The triplicate samples are placed in separate desiccators in order to avoid complete loss in case of an accident to one desiccator. When full the desiccators are exhausted to within a one-centimeter vacuum by means of a suitable vacuum pump. The desiccators are carefully rotated four or five times daily in order to mix the concentrated sulphuric acid with the supernatant aqueous layer. After about 48 hours or longer, as convenient, the tubes are transferred to desiccators containing fresh acid, and the drying is continued for three or four days or even a week; at the end of which

time the tubes are weighed and dried again for about two days over fresh acid. Repeated weighings should check within 1 to 2 mg. In opening the desiccators air is allowed to bubble slowly through sulphuric acid into the desiccator until the vacuum is entirely destroyed.

The A. O. A. C. methods follow this general scheme. They stipulate that the sulphuric acid should be boiled for 4 hours in a large Kjeldahl flask, then the mouth of the flask should be closed with a stopper carrying a calcium chloride tube and the acid allowed to cool. When the sample is not to be used for subsequent fat determination, a metal dish, 5–10 cm. in diameter and provided with a tightly fitting cover, may be used to receive the sample. The cover should be removed during drying.

*Distillation Method.*—Bidwell and Sterling (*J. Assoc. Off. Agr. Chem.*, 1925, **8**, 295) describe a method for the direct determination of moisture which presents an improvement over present methods in accuracy and speed. They employ a modification of the tube used by Dean and Stark (*J. Ind. Eng. Chem.*, 1920, **12**, 486). Marcusson (*Mitt. k. Materialprüfungsamt*, 1905, **23**, 58) had previously described a method employing xylene, and Rogers (*U. S. Dept. Agr., Bur. Chem., Bull.* **137**, p. 172) had applied this to leather, but substituted toluene for the immiscible volatile liquid. The directions of Bidwell and Sterling follow:

Introduce into a 250 c.c. Pyrex Erlenmeyer flask sufficient sample to give 2–5 c.c. of water. If the material is likely to bump, add enough dry sand to cover the bottom of the flask. Then add sufficient toluene to cover the sample completely, usually about 75 c.c., and connect the apparatus as shown and described in the original paper. Fill the receiving tube with toluene by pouring through the top of the condenser. Heat the contents of the flask to boiling and

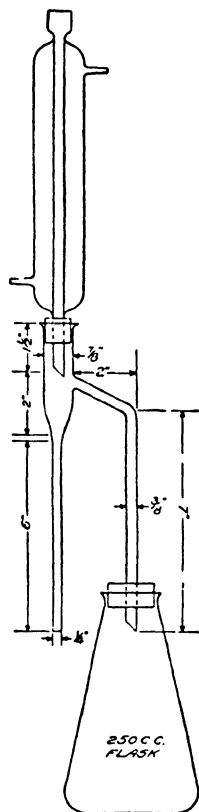


FIG. 20.—Diagram of apparatus for moisture determination by distillation method according to Bidwell and Sterling. (By courtesy of the *Journal of the Association of Official Agricultural Chemists*.)

distil slowly, about 2 drops per second, until most of the water has passed over. Then increase the rate of distillation to about 4 drops per second. When the water is apparently all over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distil. If it does, repeat the washing-down process. If any water remains in the condenser, remove it by brushing down with a tube brush attached to a copper wire and saturated with toluene, washing down the condenser at the same time. The entire process is usually completed within one hour. Allow the receiving tube to come to room temperature. If any drops adhere to the sides of the tube, they can be forced down by a rubber band wrapped around a copper wire. Read the volume of water and calculate to percentage. The tube is calibrated in tenths of a c.c., and the column can be read to hundredths with reasonable accuracy. It is necessary to have the condenser and receiving tube chemically clean in order to prevent an undue quantity of water sticking to the condenser and drops of water adhering to the sides of the receiving tube. Clean with chromic-sulphuric acid, rinse with alcohol, and dry in an oven.

The method was tested by adding 1 c.c. of water to the flask, and it gave a reading of 0.98 c.c. in the receiving tube. A second addition of 1 c.c. of water gave a new reading of 1.98 c.c. Successive additions of 1 c.c. gave readings of 2.98 c.c. and 3.98 c.c. In this way the tube may be standardised. The tube consists of a 5 c.c. Mohr's pipette sealed on one end and attached to the apparatus at the other end. The method has been tested with a number of products. Sterling (*J. Assoc. Off. Agr. Chem.*, 1926, **9**, 153) gives a report on the use of the method by four different analysts. The results obtained were very satisfactory.

**Fat.**—Crude fat or ether extract may be determined on the residue from the moisture determination if the extraction shells or tubes have been employed. If not, samples may be weighed out, placed in extraction shells, dried to constant weight, and then used for the extraction.

The dried samples are extracted for 16–24 hours with anhydrous ether in Soxhlet extractors or similar apparatus. After extraction the tubes may be partially dried in an electric oven at a low temperature, proper precautions being observed against possible ignition of the ether fumes. After this partial drying, the drying is completed

in vacuum desiccators as for the moisture determination. The loss in weight is due to fat.

*The A. O. A. C. methods* prescribe drying the extract at the temperature of boiling water for 30 minutes, cooling in a desiccator, and weighing. This is continued at 30-minute intervals until the weight is constant.

*According to Richardson*, it is difficult to extract all the fat from muscular tissue by the ordinary methods, and consequently more elaborate methods have been devised, such as alternate extraction with a volatile solvent and digestion of the residue with pepsin. But such a complicated method as this introduces as many errors as it obviates, so that for all ordinary work a simpler procedure, such as the following, gives results which are quite satisfactory.

The residue from the moisture determination by one of the vacuum methods, or a sample (2-10 grm.) weighed and dried *in vacuo* for the purpose, is ground in an agate mortar with sand which has been purified in the usual way. The ground mixture is transferred to a Schleicher and Schüll extraction shell, or an alundum (Norton Company, Worcester, Mass.) extraction thimble, and extracted for 16 hours or longer in a Soxhlet or other suitable form of continuous extraction apparatus, with petroleum spirit or absolute ethyl ether. It is sometimes advisable to remove the residue from the shell at the end of the extraction period, regrind it with sand, and re-extract it. The ether is evaporated from the extract on the steam-bath and the last traces of solvent removed by heat in an oven, preferably in a vacuum oven to avoid oxidation. The residue is then cooled as usual in a desiccator and weighed. It is always well to examine the dried fat for solubility in petroleum spirit. Insoluble impurities should be filtered off, well washed, and the fat again dried *in vacuo* and weighed.

The ordinary fat solvents dissolve from meat not only fats but also lecithin, and sometimes dextrin and other carbohydrates. In ordinary work these impurities are present in small quantity and do not seriously affect the result.

For other methods of fat estimation in animal tissue consult *Abderhalden, Handbuch der biochemischen Arbeitsmethoden* (Berlin, 1910), 2, 238 (good bibliography) and more recent editions.

*Separation of Fat for Further Examination.*—This can be accomplished in several ways. If the fatty tissue can be separated by



means of the knife, it should be hashed and rendered in a porcelain dish at as low a temperature as possible, on the steam-bath or on wire gauze over a flame. Or the fat may be extracted by shaking the hashed tissue at intervals in a flask for a long period with ether or petroleum spirit. The criticism of this method is that extraction may not be complete and the softer portions of the fat tend to dissolve first. A third method consists in extracting the tissue in the same way as for an analysis, but on a larger scale. After separation the fat is examined according to the methods for fat analysis (see Vol. II). Estimations should never be made on the fatty tissue but only on the separated fat (*J. Amer. Chem. Soc.*, 1910, 32, 568).

**Ash.**—The Missouri directions call for the use of 10–15 grm. samples (the larger samples for fatter meats) which are weighed out by difference into porcelain crucibles. The samples are dried in ovens and then charred carefully. Then they are ashed over Fletcher burners, using a low heat and taking plenty of time. In this way fusion and loss of chlorides is prevented.

According to Richardson the direct ignition of the meat to an ash is never desirable. He recommends using the dried sample of meat, or a convenient portion of it, or a specially dried sample (see Missouri method) which is charred in a porcelain, silica, or platinum dish. In the experience of the author continuous use of platinum with such materials as meat eventually ruins the platinum dish, owing to a reduction of the phosphorus of the flesh by the charred organic matter, with the formation of a compound of platinum and phosphorus which is very friable. Continuing Richardson's directions, the charred mass is extracted by digestion with hot water, the extract filtered off through an ashless filter paper, the char thoroughly washed with hot water and returned with the filter paper to the original dish and ignited to a white ash. The extract is then evaporated to dryness in the same dish, dried at 100°–105°, very gently ignited below a dull red heat, cooled, and weighed. The method is especially recommended when sodium chloride is present in any quantity or when the ash shows a tendency to fuse.

*The A. O. A. C. method* is similar to Richardson's.

**Total Nitrogen.**—The author strongly recommends the Kjeldahl-Gunning-Arnold method in all cases. Sulphuric acid, sodium (or potassium) sulphate, and mercury are all necessary in order to obtain a higher boiling point for the acid and more ready oxidation

of the sample. With samples containing much fat it is especially necessary to avoid less thorough methods or the new short digestion methods. The use of copper sulphate, so desirable with urines and similar easily digested substances, is not to be recommended.

Samples are weighed out from weighing bottles and placed in a good filter paper, such as S. and S. No. 595, and then introduced into a Kjeldahl flask. A small flask, 500 c.c., may be used for lean meats and other small samples, but an 800 c.c. flask is preferable for fatter samples and is preferred by many workers in all determinations. For hide and hair 0.50 to 0.75 grm. is used, for lean meat 1.00 to 1.25 grm., and for fat samples 2.50 to 3.50 grm.; other samples in accordance with the nitrogen content. Twenty-five c.c. of C. P. concentrated sulphuric acid are used for the meats, and 35 to 50 c.c. for fats. About 0.7 grm. of mercury is added and the digestion is made on a digestion frame. When the sample has ceased foaming and is not pasty, 7 to 10 grm. of potassium or sodium sulphate are added and the digestion is continued for one or two hours. The flasks are then cooled, the necks washed down with water, and the digestion renewed for an hour or more. About 300 c.c. of nitrogen-free water are added to the cool flasks, also a piece of paraffin wax the size of a pea and a few small pieces of granulated zinc. Then 85 c.c. of the alkali solution (100 c.c. for fats) are added carefully, the flask is connected with a condenser, the contents are mixed, the flasks boiled for 40 minutes, and the distillate caught in a wide-mouthed receiving flask containing the necessary amount of  $N/10$  hydrochloric acid with some cochineal indicator. The above alkali solution is made by dissolving 40 pounds of a high grade commercial alkali and 375 grm. of potassium sulphide in 30 litres of distilled water. The solution keeps well. If a smaller quantity is desired the same relative proportions should be used.

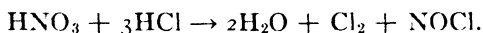
Richardson recommends continuing the digestion for 5 hours and points out that the appearance of the digest does not afford a positive criterion as to the completion of the process.

Shedd (*J. Assoc. Off. Agr. Chem.*, 1927, **10**, 507) recommends rapid boiling of the sulphuric acid digest as an aid to a shortened period of digestion. He employs 25 c.c. of sulphuric acid, 18 grm. of dry sodium sulphate, 0.7 grm. of mercury (or 0.25 grm. copper sulphate) and boils rapidly over a Fisher burner for 20 minutes. In 10 minutes the digest is clear, and 10 minutes are added for

safety. He has compared this method with the 5-hour digestion over a slow burner and has used many samples of different materials.

McMillan (*J. Assoc. Off. Agr. Chem.*, 1928, **11**, 408) has applied the short digestion method to meats. He uses 3 grm. of meat, 35 c.c. of sulphuric acid, 15 grm. of powdered potassium sulphate, and 0.7 grm. of mercury in an 800 c.c. Kjeldahl flask. The flask and contents are heated over a Gilmer electric heater which had previously come to full heat. Digestion is continued for 30 minutes—20 minutes to a colourless state and 10 minutes added for safety. When cool, 500 c.c. of water, 2 grm. of powdered talc, and 85 c.c. of alkali sulphide solution are added. The latter consists of 25 c.c. of a 4% solution of sodium sulphide ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ) and 60 c.c. of a solution of sodium hydroxide, sp. gr. 1.45. This method was compared with the Gunning method and found satisfactory. A comparison with the Kjeldahl-Gunning-Arnold method was not made. Kerr (*J. Assoc. Off. Agr. Chem.*, 1928, **11**, 507) recommends that the shorter time of digestion required by this rapid-boiling short-digestion method be recognised in the official methods. The present author believes this is premature until a careful comparison has been made with the Kjeldahl-Gunning-Arnold method.

In using this method for total nitrogen in meats, or the method for total nitrogen in the water extract, it should be remembered that meats containing nitrates and salt require special treatment. Richardson (*J. Amer. Chem. Soc.*, 1908, **30**, 421) has pointed out that modifications of the Kjeldahl method to include nitric nitrogen are inapplicable to cured meats, since the sulphuric acid acts on the salt and nitrate to give hydrochloric and nitric acid. Before the latter is reduced to ammonia the following reaction occurs



For cured meats, meat extracts, or aqueous extracts from cured meats, Richardson recommends determining the nitric nitrogen by the Schloesing-Wagner method, and in another portion determining the nitrogen, exclusive of nitrates (and nitrites), by adding to the substance in the Kjeldahl flask 10 c.c. more or less of saturated ferrous chloride solution and boiling with dilute sulphuric acid until the nitrates are destroyed. Otherwise the total nitrogen will include some, but not all, of the nitric nitrogen.

**Protein.**—The total crude protein may be calculated from the total nitrogen by means of the standard factor, 6.25. This factor

is derived from the average percentage of nitrogen found in animal proteins. Armsby and Moulton (*The Animal as a Converter of Matter and Energy*, Chemical Catalog Co., New York, 1925, p. 54) have discussed this question. The nitrogen in the total ash- and fat-free dry matter of the animals, the chemical analysis of which was there reported, averaged 15.97%. The nitrogen content of animal proteins is about 16.06%. These authors go on to show that in the lean meat of animals the fat- and ash-free dry matter contains from 16.16 to 16.90% of nitrogen. For the proteins of lean flesh the factor of 6 would appear to be more justified than the conventional 6.25. In all cases the factor employed should be stated. For the present, it would seem best to adhere to the usual conventional factor.

**Volatile Acids and Volatile Sulphur Compounds** (sulphur dioxide, hydrogen sulphide, hydrosulphides, thio-ethers).—One hundred grm. of the hashed sample are weighed into an evaporating dish containing 200 c.c. of distilled water and, after stirring, the mixture is poured into a 1,000 c.c. distilling flask. The dish is rinsed with 100 c.c. of water. This procedure allows a thorough breaking up of the sample and obviates sticking during distillation. One c.c. of syrupy phosphoric acid is added, and distillation is carried on in a current of steam. The distillate is condensed by means of a Hopkins condenser and collected in a 500 c.c. flask containing 10 c.c. of sodium hydroxide solution, by means of a tube running to the bottom of the flask. The excess of sodium hydroxide is titrated back with  $N/2$  hydrochloric acid, phenolphthalein being used as indicator. Bromine is then added to oxidise sulphur compounds, the solution taken to dryness in a porcelain dish, and the residue ignited until carbon is burned off. The residue is taken up with hydrochloric acid and water, and the sulphates estimated by means of barium chloride in the usual way.

**Total Sulphur.**—For methods of estimating sulphur see Abderhalden, *Handbuch der biochemischen Arbeitsmethoden*, 1910, 3, 794. Schreiber, *Circular 56*, U. S. Dept. Agric. Bur. Chem., 1910.

**Total Phosphorus.**—Fifty grm. of the sample are weighed into a 250 c.c. beaker, and 50 c.c. of a mixture of 6 parts of nitric and 1 part of hydrochloric acid are added. The resulting solution is taken nearly to dryness (10–15 c.c.) on the hot plate, taken up with hot water, and made up to volume after cooling, in a 250 c.c. flask.

The solution is filtered, and portions of 100 c.c. are taken in duplicate for the estimation. This solution is precipitated with the ordinary acid ammonium molybdate solution, and the precipitate dissolved in ammonia and reprecipitated with nitric acid and a little of the molybdate solution. The precipitate is filtered off, dissolved in ammonia and precipitated as usual with magnesia mixture, and the precipitate weighed in a Gooch crucible as magnesium pyrophosphate.

According to the Missouri directions, the crucibles from the ash determinations are leached with strong hydrochloric acid and a little nitric acid. The solutions are neutralised and ammonium nitrate is added. The phosphorus is precipitated at  $65^{\circ}$  with acid ammonium molybdate. The yellow phospho-molybdate is filtered off, washed, dissolved in ammonia and hot water, and the phosphorus is reprecipitated with magnesia mixture. The precipitate is ignited strongly in a gasoline muffle and weighed as the pyrophosphate.

**Soluble Phosphorus.**—Richardson has proposed the following method which is based on the assumption that all the soluble phosphorus is in the inorganic form. The method really determines the total soluble phosphorus. One hundred grm. of meat are extracted successively in a porcelain dish with four 250 c.c. portions of boiling distilled water and filtered through a folded filter. The last portion is squeezed through cheese-cloth and the filter and contents washed with boiling water. Two portions of 500 c.c. each are evaporated to about 100 c.c. Thirty c.c. of nitric and 5 c.c. of hydrochloric acid are added, and the volume reduced on the hot plate to 10–15 c.c. When oxidation is at an end, hot water is added and the phosphorus determined as described under total phosphorus.

In determining inorganic phosphorus in meat and eggs, Chapin and Powick (*J. Biol. Chem.*, 1915, 20, 97) extract the inorganic phosphoric acid by using approximately  $N/10$  hydrochloric acid with an excess of picric acid, the estimation being made in aliquot portions of the filtrate. In case the amount of water in the sample is not known and the volume of the material insoluble in the extracting liquid cannot be neglected, a known amount of potassium iodide may be added as a "marker," and from the concentration of the potassium iodide in the extract the degree of dilution by the water in the sample may be calculated. The potassium iodide is deter-

mined by Schirmer's nitrous acid and urea method (*Arch. Pharm.*, 1912, **250**, 448). The phosphoric acid is first precipitated with magnesia mixture and then as ammonium phosphomolybdate by Lorentz's method.

For methods of determining the soluble inorganic phosphorus separately from the soluble organic phosphorus see the methods of analysis of aqueous extracts of meat which follow.

Vladesco (*Bull. Soc. Chim. Biol.*, 1929, **11**, 986) suggests a method for determining the forms of phosphorus in animal tissues. He observes that fresh animal tissues, dissolved by heating in concentrated nitric acid until clear, set free their inorganic phosphates which can be directly precipitated. Further digestion with the addition of sulphuric acid gives the total phosphate. He reports analyses for the various organs of the ox, horse, and dog.

**Phosphagen.**—Eggleton and Eggleton (*J. Physiol.*, 1929, **68**, 193) propose the following method as the most rapid and direct available for the determination of phosphagen. Three stock solutions: (a) 5.5*N* sulphuric acid, (b) 5% ammonium molybdate, and (c) 0.5% hydroquinone in 15% sodium thiosulphate ( $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ ), are freshly mixed in the proportions of 2 parts of (a), 2 of (b), and 1 of (c). Five c.c. portions of this reagent are added simultaneously to a standard phosphate solution and to the trichloroacetic acid extract of a muscle (both containing about 0.08 mg. of directly determinable phosphorus and having a volume slightly less than 10 c.c.), the volumes are rapidly adjusted to 15 c.c., and the solutions are mixed. By reading the standard against the unknown in the colorimeter at 2, 4, and 6 minutes, the readings, plotted against time, form a straight line and can be extrapolated backward to zero time with a ruler. The method is accurate provided the ratio of orthophosphate to phosphagen phosphate is less than 6 and greater than 0.5, but cannot be used with confidence for well-oxygenated resting muscles or for very fatigued muscles. A new method is described for the separation and separate determination of the orthophosphate and phosphagen in 0.5 to 1.0 grm. of muscle based on the different solubilities of the barium salts. The method can be extended for the additional determination of pyrophosphate and of two distinct fractions of phosphoric esters with soluble and insoluble barium salts, respectively. Resting voluntary muscle showed a distribution of acid-soluble phosphorus as follows: orthophosphate,

15%; pyrophosphate, 20%; phosphagen, 50%; acid-soluble phosphoric esters with soluble barium salts, 4%; and esters with insoluble barium salts, 11%.

**Creatine and Creatinine.**—The methods of determining creatine and creatinine in meat are discussed on pp. 299, 497, 507. A method of determination in the original meat is given on pp. 272, 301.

### Cold-water Extract

For the investigation of the soluble substances present in meat, it is customary to work on a cold-water extract or on the juice of the lean portions. Cold water does not extract all of the soluble matters, since the juice contains a considerably higher portion of them than does a cold-water extract made according to any of the usual methods. The failure of water to extract all the soluble matters may be partly due to the indiffusibility or the slow diffusibility of the soluble proteins through the sarcolemma of the muscle fibres. The globulin of meat is, of course, not soluble in pure water. A water extract dissolves some globulin on account of the inorganic salts of the meat which are dissolved. The use of various salts in making extracts causes solution of considerably more protein, since such a procedure removes globulin. The amount removed seems to depend on the freshness of the meat, as well as on the concentration and kind of salt used.

The cold-water extract method was proposed by König (*Chemie der menschlichen Nahrungs- und Genussmittel*, 3), was developed and used extensively by Grindley (*J. Amer. Chem. Soc.*, 1904, 26, 1086; 1905, 27, 658; 1906, 28, 25 and 468), and was further used by Trowbridge, Francis, Moulton, Haigh, and Ritchie at Missouri (*J. Ind. and Eng. Chem.*, 1910, 2, 212; *J. Biol. Chem.*, 1910, 8, 481; *Missouri Agr. Expt. Sta., Research Bull.* 59, 1922).

The method, as used at Missouri, is as follows: Of the lean meats, exactly 120 gm. are weighed out in three portions, or 180 gm. of fat samples in four weighings, and distributed into twenty 100 c.c. Jena or Pyrex beakers in approximately equal amounts. The beakers are numbered and the division of the different portions indicated, so that if a beaker is broken it is not necessary to reweigh the whole sample, but only that portion from which the loss occurred. Fifty c.c. of recently boiled, cold, nitrogen-free water are measured out, and the portion of meat in beaker No. 1 moistened with about

5 c.c., then mixed with a stirring rod to a pasty condition; more water is added and mixed until the whole 50 c.c. have been added. This operation is repeated for each of the 20 beakers. After standing about one-half hour with frequent stirring, the extract is poured on to 11 cm. (S. and S. No. 595) filters and filtered into 300 c.c. flasks without permitting the major portions of the residue to flow from the beakers. If, during the process, any considerable amount of meat residue collects upon the filter, it is returned to the corresponding beaker with the aid of a stirring rod. Next, 25 c.c. of the water are added to the residue in each beaker, and the mixing and filtering continued after the first liquid has drained through the filter. This is repeated until eight 25 c.c. portions have been used. With the last water the residue in the beaker is transferred to the filter and the beaker and filter is washed twice with 10 c.c. portions of water. The volume of filtrate is thus about 270 c.c. The filtrates are combined, the flasks rinsed, and the volume of the extract is made up with water to 6 litres. The extract is then carefully mixed, aeration being avoided, and filtered through a dry filter paper.

The A. O. A. C. methods (*Official and Tentative Methods of Analysis*, p. 243) call for the use of 7–25 gm. of sample (depending upon the water content) weighed into a 150 c.c. beaker, using, as above, 50 c.c. of water and stirring during 15 minutes. The liquid is filtered into a 500 c.c. volumetric flask. Three successive portions of 50 c.c. each are then used, followed by four 25 c.c. portions, with final washing on the filter with three 10 c.c. portions of water. The filtrate is then diluted to the mark and mixed thoroughly.

Richardson and Scherubel (*J. Amer. Chem. Soc.*, 1908, **30**, 1515) have modified the method. The following simplified procedure has been found satisfactory by Richardson, yielding results which agree with those obtained by the more complicated procedure:

Duplicates of 100 gm. each of the finely hashed lean meat, are weighed into 8 in. porcelain dishes, and 250 c.c. of cold distilled water added. The mass is macerated, with occasional stirring, preferably in a room at low temperature (in summer 2–5°). If a chill-room is not available, the temperature should be kept down by means of ice. The extract is then filtered through linen or cheese-cloth of suitable mesh, placed in a funnel, and the meat wrung out by hand in the cloth. The meat is then returned to the dish, and the process repeated with smaller amounts of water 5 times or



until a litre of extract is obtained; or 4 portions of about 250 c.c. may be used. If the method is carefully followed, only negligible amounts of extractive are obtained by a second extraction. The extract obtained is filtered for all determinations.

The juices which exude from frozen meat (especially slowly frozen meat) on thawing are rich in soluble constituents. It is sometimes desirable to examine such meat juice. The following method of preparation is useful:

*Technique for Obtaining Meat Juice.*—The meat, which should be in the form of lean pieces of moderate size (1–10 lb.), is first frozen hard. The frozen meat is brought to the laboratory and, without thawing, is sawed and chopped into blocks about 1 inch of an edge. These pieces are then wrapped in linen or muslin, and firmly tied. Pressure is applied to the meat in a colander or other container with perforated bottom, by means of weights or in a screw press. The pressure is continued for a length of time which varies from a few hours to 24 hours. It is advantageous to remove the meat after the juice has ceased to run freely, grind it in an "Enterprise" hasher, and then return it to the press. At least 20% of juice should be obtained. The pressing should, of course, be done in a cool place, and the juice collected in such a way as to avoid evaporation of water. The freezing of the meat appears to change the sarcolemma into a sieve-like structure which permits the passage of colloidal substances. After thawing, the original semi-permeable structure is resumed, so that a cold-water extraction of frozen and thawed meat removes no more solids than an extraction of unfrozen meat.

### **Methods for the Analysis of Cold-water Extracts Applicable Also to Meat Juice**

It is convenient in selecting amounts of extract for the various determinations to select a volume which represents an even weight of the original meat. Thus, if the Missouri directions are followed, 100 c.c. of extract of lean meat will represent 2 grm. of original meat. In the case of fat meats 3 grm. are represented by 100 c.c. All calculations are made on the basis of the original meat.

**Total Solids.**—One hundred c.c. portions are evaporated to dryness in weighed platinum, silica, or porcelain dishes, on the steam bath and then dried to constant weight in an oven at 100° to 105°. One

hour's drying is sufficient. A vacuum oven is preferred by Richardson.

**Ash.**—The dried residues (if platinum dishes have been used according to the Missouri directions) are carefully ignited over a free flame at a low red heat until colourless. The dishes are then quickly weighed, heated, and weighed again, and the procedure repeated until a constant weight is obtained.

Richardson prefers to char the residue, extract the charred mass with hot water, filter on an ashless filter, and wash with hot water. The filter paper and contents are returned to the original dish and ashed; the filtrate is added and evaporated to dryness on the steam bath. The residue is then dried in the oven, cooled and weighed.

**Total Nitrogen.**—In the Missouri method 100 c.c. of extract are used, whilst the Richardson and A. O. A. C. methods call for 50 c.c. The portions are transferred to Kjeldahl flasks and the total nitrogen is determined by the Kjeldahl-Gunning-Arnold method. The A. O. A. C. permits the use of other standard methods. When applied to meats containing nitrate or nitrite, this method, of course, does not include nitrogen in those forms.

**Nitrogen in Coagulable Proteins.**—There are several modifications of a general method of coagulating the proteins. The most important will be described.

*Missouri Method.*—Portions of 100 c.c. are evaporated to about 25 c.c. in the presence of freshly precipitated magnesium carbonate in order to maintain neutrality during the evaporation. When a volume of 25 c.c. has been obtained, the coagulum is filtered off and washed with hot water. The residue on the filter is then transferred to a Kjeldahl flask and the nitrogen is determined.

*Richardson Method.*—A 50 c.c. portion of the extract is transferred to a 100 c.c. beaker and concentrated on the steam-bath to one-half the volume. The precipitate is filtered off and washed with hot water. The filtrate is made neutral to litmus paper with  $N/10$  sodium hydroxide. If any precipitate forms upon further heating, it is filtered off and washed with hot water. This second precipitate is often separately estimated as "syntonin." The two precipitates and filter papers are transferred to a Kjeldahl flask and the nitrogen determined, as given above under "Total Nitrogen," the distillate being received in  $N/10$  sulphuric acid.

*A. O. A. C. Method.*—This method is similar to the preceding one. One hundred and fifty c.c. of the extract are evaporated in a 250 c.c. beaker to 40 c.c. on a steam-bath, with occasional stirring. It is then neutralised to phenolphthalein, 1 c.c. of 0.1 *N* acetic acid added, and the liquid boiled gently for 5 minutes. The coagulum should settle at once, leaving a clear liquid. This is filtered through a quantitative paper and the beaker washed thoroughly 4 times with hot water, special care being taken to clean the sides. Finally the coagulum is washed on the filter 3 times. The filtrate and washings are diluted to a definite volume and reserved for the determination of proteose, peptone and gelatin, and creatine. The coagulum is transferred with the filter paper to a Kjeldahl flask and any of the material adhering to the beaker tendered with concentrated sulphuric acid, the usual 25 c.c. of acid in 5 c.c. portions being taken for this purpose, the acid being heated in the beaker on a hot plate, and the sides rubbed with a glass rod. By this means any coagulum remaining in the beaker or adhering to its side is dissolved. The acid is then transferred to the Kjeldahl flask containing the filter paper and coagulum, and the determination is completed as usual.

**Nitrogen in Albumoses and Proteoses.** *Missouri Method.*—The filtrate and washings from the determination of coagulable nitrogen are evaporated, after slight acidification with acetic acid, to a volume of 30 c.c. on the steam-bath. After cooling, 1 c.c. of 50% sulphuric acid is added, and the solution is completely saturated with zinc sulphate. It is then heated on the steam-bath with stirring until clear, allowed to stand for 12 hours, and filtered. The precipitate is thoroughly washed with a saturated solution of zinc sulphate slightly acidified with sulphuric acid. Nitrogen in the precipitate is determined as usual.

Moulton (*J. Assoc. Off. Agr. Chem.*, 1922, 6, 83) has shown that there is probably a relation between the proteins to be precipitated and the acidity of the solution. With larger amounts of coagulable material more acid seems to be required to give complete precipitation, a solution containing about 15 mg. of coagulable nitrogen requiring 5 c.c. of dilute (1 to 1) sulphuric acid, whilst solutions containing 6 or 7 mg. were completely precipitated with 1 c.c. of the acid.

*Richardson's Method.*—Richardson's directions are essentially the same. He states that about 35 grm. of finely powdered zinc sulphate are needed per 50 c.c. of liquid.

**Nitrogen in Peptones and Peptids.**—Salt and tannic acid precipitate all protein material, leaving amino acids and extractives. It is not convenient to determine the nitrogen in the coagulum, and so the filtrate is used for the determination of amino acid and extractive nitrogen as shown below. From the total water-soluble nitrogen is subtracted the sum of the coagulable, the nitrogen precipitated by zinc sulphate, and the amino acid and extractive nitrogen. The difference will be the peptones and peptids. Other nitrogen-containing materials, such as creatine, are partly precipitated by the tannin salt method.

**Amino Acid and Extractive Nitrogen.**—This method, known in its present form as the “tannin-salt” method, was proposed by Schjerning (*Z. anal. Chem.*, 1900, **39**, 545), and further developed by Bigelow and Cook (*U. S. Dept. of Agric. Bur. Chem. Bull.* **73**; *J. Amer. Chem. Soc.*, 1906, **28**, 1485) for the determination of peptones in meat extracts. As applied to the cold-water extract of meat, it is conducted as follows: 20 c.c. of the cold-water extract are placed in a 100 c.c. volumetric flask, 50 c.c. of a saturated salt solution added, and then 30 c.c. of a 24% tannic acid solution. This tannic acid must be of highest possible purity. The precipitation is made in a cold room (or ice-box) not above 12°, and the flask is allowed to remain there overnight. The next day the solution is filtered, and the nitrogen determined in 50 c.c. of the filtrate. The figure thus obtained is multiplied by 2. A blank is made on 30 c.c. of the tannic acid solution, and the nitrogen found deducted from that found in the filtrate ( $\times 2$ ). This figure, subtracted from the total nitrogen in the extract, gives the amount precipitated by the tannic acid. The tannic acid nitrogen, minus the coagulable nitrogen, gives the albumose and peptone nitrogen. In the writer's experience this method is difficult to use and to be made to yield concordant results. The “personal equation” appears to influence results, and there is a positive and uncertain source of error in the nitrogen present in the purest tannic acid obtainable. Oftentimes the nitrogen introduced with the tannic acid is much greater than the amount to be determined. If a nitrogen-free tannic acid were obtainable, the results would doubtless be much more satisfactory. Where the amount of albumoses and peptones to be determined is small, as in the cold-water extract or juice from fresh meat, only a nitrogen-free tannic acid could make the method a satisfactory one

to use. The method is, of course, more satisfactory for use in the analysis of meat extracts and commercial peptones.

*Missouri Method.*—The Missouri directions are essentially the same as above. One hundred c.c. of the original water extract, after the removal of the heat coagulable nitrogen, are used. The filtrate and washings, after concentration, are transferred to 100 c.c. graduated flasks. Fairly wide necks are an advantage. To this are added 15 grm. of sodium chloride and 30 c.c. of a 24% solution of tannic acid. The flasks are filled to the mark, shaken vigorously, and allowed to stand over-night at a temperature of 15°. Of course, the material in the flask must be at the same temperature. The next day the solutions are filtered through dry folded filters, and the nitrogen determined in 50 c.c. of the filtrate. Blanks must be run to determine the nitrogen in the reagents. It is well to repeat that the method is not completely satisfactory for reasons set forth in the above paragraph.

*A. O. A. C. Method.*—By these methods the proteose and albumose nitrogen are not determined separately. A 50 c.c. aliquot portion of the filtrate from the heat-coagulable nitrogen determination is used, as in the Missouri method, but 12° is specified as the proper temperature. The 50 c.c. aliquot portion is acidified with a few drops of sulphuric acid, and the solution is evaporated on a steam-bath to dryness, with the use of a vacuum pump. This is a good procedure, since by the Missouri method foaming is very apt to occur.

**Nitrogen in Meat Bases.**—When nitrates are absent, the filtrate from a second coagulable determination is taken and the total nitrogen determined therein by the usual method, as given above. When nitrates are present they are removed, after separating the coagulable proteins, by adding 10 c.c. of saturated ferrous chloride (freshly made) and 5 c.c. of strong hydrochloric acid, and boiling a sufficient length of time. The usual total nitrogen determination is then made. The "meat-base" nitrogen is obtained by subtracting from this amount (albumose + meat-base nitrogen) the albumose nitrogen. If it is desired to calculate to the meat-bases, the factor 3.12 may be used.

*A. O. A. C. Method.*—By this method one subtracts from the total nitrogen in the meat the sum of the insoluble nitrogen, the coagulable nitrogen, and the proteose, peptone, and gelatin nitrogen. *It should be noted that any gelatin present will be precipitated by the salt and*

**tannic acid.** The result is multiplied by 3.12 to obtain the meat bases. Or the result may be left expressed in terms of nitrogen in the meat bases. The Missouri method calls this fraction "amino acid and extractive nitrogen."

**Creatine and Creatinine.**—Of the various meat bases present in meats or meat extracts, those most readily determined by a simple practical method are creatinine and creatine. Some suggested methods, in addition to the one given here, will be found under MEAT EXTRACTS.

In 1904 Folin (*Z. physiol. Chem.*, 1904, 41, 223; *Amer. J. Physiol.*, 1905, 13, 48) proposed a method for estimating creatinine in urine, based on the orange-yellow colour produced by the action of creatinine on picric acid in alkaline solution (Jaffé reaction). Grindley (*J. Biol. Chem.*, 1907, 3, 491) has applied the method to meat extracts and the cold-water extract of meat.

**A. O. A. C. Method.**—This method follows the older Folin procedure. Evaporate an aliquot portion of the water extract or the remaining portion of the filtrate and washings from the coagulable nitrogen (a portion having been used for the determination of proteose, peptone, and gelatin nitrogen) to 5–10 c.c. Transfer with a minimum quantity of hot water to a 50 c.c. volumetric flask, keeping the volume below 30 c.c., add 10 c.c. of 2 *N* hydrochloric acid and mix. Hydrolyse in an autoclave at 117°–120° for 20 minutes, allow the flask to cool somewhat, remove, and chill under running water. Partly neutralise the excess of acid by adding 7.5 c.c. of 10% sodium hydroxide solution free from carbonates. Dilute to the mark and mix. Make a preliminary reading on 20 c.c. with a Duboscq colorimeter to ascertain the volume to use to obtain a reading of approximately 8 mm. Transfer such a volume of the solution to a 500 c.c. volumetric flask and add 10 c.c. of 10% sodium hydroxide solution and 30 c.c. of saturated picric acid solution (1.2%). Mix and rotate for 30 seconds and let the flask stand for exactly 4.5 minutes. Dilute to the mark at once with water, shake thoroughly, and read in a Duboscq colorimeter, comparing the colour with 0.5 *N* potassium dichromate solution set at 8 mm. If the reading is too high or too low (above 9.5 or below 7 mm.), calculate the quantity necessary to obtain a reading of about 8 mm. The strength of the dichromate solution used must be checked against a standard creatine solution. To obtain the values, divide 81 by

the reading and multiply by the volume factor to obtain the mg. of creatinine. This value, multiplied by 1.16, gives creatine, which divided by the weight of the sample and multiplied by 100, gives the percentage of creatine.

To obtain the pre-formed creatinine, transfer the aliquot part to be used to the 500 c.c. volumetric flask, add the picric acid and sodium hydroxide, and proceed as above. Subtracting the percentage of creatinine found from the result obtained in the above determination gives the creatine.

The method, as originally applied by Grindley (*J. Biol. Chem.*, 1907, **2**, 309) and others, was criticised by Hehner (*Pharm. J.*, 1907, **78**, 683) on several grounds. The conversion of creatine to creatinine in the autoclave is due to Benedict and Myers (*Amer. J. Physiol.*, 1907, **18**, 397). Few chemists have been able to obtain consistent results by this method. Chapman (*Proc. 7th Internat. Cong. App. Chemistry*, London, 1909) has shown that the red colour in Jaffé's reaction is not due to the formation of creatinine picrate, but to the reduction of picric acid in alkaline solution to a mixture of amino-dinitrophenol and diamino-nitrophenol, the alkaline salts of which are deeply coloured. The same coloration is produced by numerous reducing agents, such as nascent hydrogen, hydroxylamine, acetone, aldehyde, ammonium sulphide, etc. The colour is due to both monamino- and diamino-phenol, and solutions of the sodium salt of picramic acid (amino-dinitrophenol) could not be used for matching the colour. The factors of temperature, time, and presence of dextrose are shown to influence the result.

Wieland (*Konserven-Z.*, **14**, 249) has pointed out that salts of acetic acid, semicarbazide, dihydroxyguanidine, and quinol give a red colour with picric acid and soda. Sudendorf and Lahrmann (*Z. Nahr. Genussm.*, 1915, **29**, 1) have made a similar observation, and have modified the method by using a 1% solution of potassium permanganate, which removes the interfering substances. After this treatment tomato juice, yeast extract, caramel, and acetone did not give the Jaffé reaction. Thompson, Wallace, and Clotworthy (*Biochem. J.*, 1913, **7**, 445) recommend creatinine picrate or the double picrate of creatinine and potassium for control of the standard dichromate solution in the Folin method. In estimating creatinine in weak solutions, the best results were obtained by using an equal quantity of *N* hydrochloric acid and heating either on the water-bath

for 3 hours or in the autoclave for 25 minutes at  $117^{\circ}$ . Identical results were obtained by both methods of heating. The optimum time and temperature for the development of the colour were 7 minutes and  $15-17^{\circ}$ . For accurate work, the readings on the colour scale are strictly proportional only if they lie between the limits of 7 and 9 mm. Dextrose to the extent of 10% does not affect the estimation of creatinine (Cf. also Baur and Trümpler, *Z. Nahr. Genussm.*, 1914, 27, 697).

*Folin Method.*—Folin himself has discarded potassium dichromate as the colour standard, preferring creatinine zinc chloride. His (*J. Biol. Chem.*, 1914, 17, 463-493) modification of the method of estimating creatine in muscle is as follows:

Transfer 5 grm. of comminuted muscle to a 200 c.c. Erlenmeyer flask and add 100 c.c. of  $N/2$  sulphuric acid. Cover the flask with tin foil and heat in the autoclave at  $130-135^{\circ}$  for 30-40 minutes. After cooling to below  $100^{\circ}$ , open the autoclave, cool the contents of the flask, and transfer to a 200 c.c. flask. Shake for a short time to break up the skeletal tissues, dilute to 200 c.c. and mix well. Filter and titrate 10 c.c. of the filtrate with 10% sodium hydroxide, using phenolphthalein as indicator. To another 10 c.c. portion in a 100 c.c. flask add 20 c.c. of saturated picric acid and enough sodium hydroxide solution to give 1.5 c.c. in excess of that required to neutralise the sulphuric acid. As standards use a solution containing creatinine equivalent to 1 mg. of creatine per c.c. (1.389 grm. of creatinine zinc chloride per litre) for striated muscle, and standards half as strong for other muscle. In the former case set the standard at 10 mm., in the latter at 20 mm. In either case, 4,000, divided by the reading of the unknown in mm., gives the creatine in mg. per 100 grm. of muscle.

*Baumann and Hines' Method.*—Baumann (*J. Biol. Chem.*, 1914, 17, 15) has reported a method of determining the total creatine in muscle which yields results, within the limits of experimental error, identical with those obtained by the method of Myers and Fine (*J. Biol. Chem.*, 1913, 14, 9) or that of Pekelharing and van Hoogenhuyze (*Z. physiol. Chem.*, 1910, 64, 262). Both of the latter methods entail the conversion of creatine into creatinine by the autoclave method of Benedict and Myers (*Amer. J. Physiol.*, 1907, 18, 397). Baumann's method, like Folin's latest one, obviates this. The method has been modified by Baumann and Hines (*J. Biol. Chem.*,



1916, 24, 439), so that the creatinine is determined by Folin's method. The details are as follows:

Fifty grm. of hashed muscle are weighed into a round-bottomed short-necked Pyrex or Jena flask. To this 125 c.c. of 5 *N* sulphuric acid and a few chips of unglazed porcelain are added, and the whole boiled for 3 hours under a reflux condenser. At the end of this time the muscle is disintegrated. The solution is now filtered quantitatively through a 15 cm. filter paper into a 250 c.c. volumetric flask, and the residue is washed thoroughly with distilled water. The fluid is cooled and the flask is filled to the mark. Then 10 c.c. of this hydrolysed, filtered extract are pipetted into a 50 c.c. volumetric flask, 9 c.c. of 10% sodium hydroxide solution are added, the solution is cooled and diluted to the mark with saturated picric acid solution, then mixed well and filtered. The creatinine is determined according to Folin. Twenty-five c.c. of the clear filtrate and the requisite amount of standard creatinine solution are pipetted into 250 c.c. volumetric flasks. Fifteen c.c. of saturated picric acid solution and sufficient distilled water to equalise the volumes in both flasks are added to the standard. The colour is developed by adding 2.5 c.c. of 10% sodium hydroxide solution to the standard and 3 c.c. to the unknown. After 10 minutes the liquids are diluted to the mark and the colours compared. The standard is set at 10 mm. The standard consists of 0.1 *N* hydrochloric acid containing the equivalent of 1 mg. of creatinine per 1 c.c. of solution. For beef 3.5 c.c., and for rabbit muscle 4.5 c.c. of this solution are required.

The above directions apply to the meat itself. In modifying them for a water extract, take an aliquot part that represents 2 grm. of meat, or a smaller quantity if the amount of creatine permits, and remove the coagulable proteins. The filtrate and washings are concentrated, transferred to a 100 c.c. flask, and made up to the mark. Of this, 10 c.c. are taken, transferred to a 100 c.c. volumetric flask, and to it are added 20 c.c. of saturated picric acid and 1.5 c.c. of 10% sodium hydroxide solution. After 10 minutes' standing it is diluted to the mark and read against a standard. The solution is highly coloured and stable. This method, which omits the heating with hydrochloric acid, gives the pre-formed creatinine.

To determine the total creatine evaporate the aliquot part representing 2 grm. of meat to 5-10 c.c., transfer with a minimum of water to a 50 c.c. volumetric flask, keeping the volume below 30 c.c.,

add 2 c.c. of 2 *N* hydrochloric acid, and mix. Hydrolyse in an autoclave at 117°–120° C. for 20 minutes, allow the liquid to cool somewhat, remove, and chill under running water. Make up to the mark, remove 10 c.c. and titrate to neutrality with 10% sodium hydroxide, with phenolphthalein as indicator. Another 10 c.c. is then measured into a 100 c.c. volumetric flask and to it are added 20 c.c. of saturated picric acid and enough 10% sodium hydroxide to give 1.5 c.c. over and above that required for the neutralisation, as shown by the titration. After 10 minutes' standing, it is diluted to the mark and read in the colorimeter.

*Application to Meat Extracts.*—In applying these methods to meat extracts, dissolve about 5 gm. of the meat extract in water, make the resulting solution up to a volume of 250 c.c., and thoroughly mix. To determine the pre-formed creatinine, take a suitable aliquot part and proceed as directed before. To obtain the total creatine, add 10 c.c. of 2 *N* hydrochloric acid and hydrolyse. Then proceed as usual.

It should be emphasised that Folin's newer modification is to be preferred to the old method. The only special apparatus required, besides the colorimeter, is a number of accurate 1-c.c. pipettes of the kind described by Folin (*J. Biol. Chem.*, 1912, **11**, 494). These are used to measure out the standard creatinine solution, which is treated exactly as the unknown (20 c.c. of saturated picric acid and 1.5 c.c. of 10% sodium hydroxide) to develop the standard colour for comparison.

**Acidity of the Extract.**—Fifty c.c. are diluted with recently boiled and cooled distilled water and titrated against *N*/10 sodium hydroxide, phenolphthalein being used as indicator. The acidity is expressed as lactic acid on the basis of the original sample. The carbon dioxide which may be present in the extract interferes with the accuracy of the method in some cases. A second estimation is sometimes made on another 50 c.c. portion after boiling  $\frac{1}{2}$  minute to remove carbon dioxide. The fact that acid is produced when proteins coagulate by heat, introduces an error which may approximately equal the one obviated.

**Total Soluble Phosphorus.**—The Missouri directions call for the use of 500 c.c. portions of the water extract, which are measured into 600 c.c. beakers and evaporated on the steam-bath to a small volume (50 c.c.). With the aid of 15 c.c. of sulphuric acid and hot

water this is transferred to 500 c.c. Kjeldahl flasks. Seven-tenths grm. of mercury and 5 grm. of sodium sulphate are added, and the extract digested as for nitrogen. When the digestion is complete, the liquid is cooled, the entire contents transferred to a 250-c.c. beaker, slightly diluted with water, neutralised with ammonia, and the procedure used for total phosphorus in meat followed.

**Soluble Organic Phosphorus.**—Hart and Andrews (*Amer. Chem. J.*, 1903, **30**, 470) were among the first to show that the phosphorus in foods was present to a considerable extent in the organic form. They attempted to remove the inorganic phosphorus from solutions by precipitation with neutral ammonium molybdate and the smallest necessary amount of nitric acid. Acid splits the organic phosphorus and thus permits it to be precipitated along with the inorganic phosphorus.

Trowbridge and Stanley (*J. Ind. Eng. Chem.*, 1910, **2**, 212) have shown that the method of Hart and Andrews fails to precipitate all of the inorganic phosphorus present. They discuss the literature, especially the work of Emmett and Grindley (*J. Amer. Chem. Soc.*, 1904, **26**, 1086; 1905, **27**, 658; 1906, **28**, 25; *J. Ind. Eng. Chem.*, 1909, **1**, 413 and 580), and show that their heat treatment of solutions of organic phosphorus change this phosphorus into the inorganic form. They then apply the method of Siegfried and Singewald (*Z. Nahr. Genussm.*, 1905, **10**, 521) which involves the precipitation of inorganic phosphorus by means of barium chloride in alkaline solution. Frances and Trowbridge (*J. Biol. Chem.*, 1910, **8**, 481) also discuss the literature and give a detailed description of the method as applied to cold-water extracts.

Take 600 c.c. portions of the extract and transfer to 1,000 c.c. Erlenmeyer flasks. Add 5 c.c. of 10% barium chloride solution, 10 c.c. of ammonia (diluted 1:1) and 45 c.c. of water. This makes a total of 660 c.c. and represents 12 grm. of the original sample of lean meat or 18 grm. of fat meat. Thoroughly mix, cover with a watch-glass and allow the flask to stand over-night or until the precipitate has settled. Filter through a dry filter and take 605 c.c. of the filtrate. This is eleven-twelfths of the total and represents 11 grm. of lean meat or 16.5 grm. of fat meat. The 605 c.c. are placed in a dry 1,000 c.c. Erlenmeyer flask, and 10 c.c. of 5% potassium sulphate and 45 c.c. of water are added, making the volume 660 c.c. The whole is thoroughly mixed and allowed to stand long

PERCENTAGE COMPOSITION OF LEAN BEEF AND WATER EXTRACTS OF THE SAME—MISSOURI METHOD

Animal No.	Age	Condition	Fresh meat				Water extract									
			Water	Fat	Ash	Phosphorus	Protein	Total nitrogen	Solids	Ash	Total phosphorus	Organic phosphorus	Soluble nitrogen	Coaguable nitrogen	Amino acid extractive nitrogen	
Lean flesh of round																
18	2½ yrs.	Thin	66.50	13.26	0.88	0.17	19.44	3.11	4.98	0.87	0.130	0.003	0.57	0.29	0.21	
121	2½ yrs.	Medium fat	69.96	8.18	0.96	0.19	20.50	3.28	5.74	0.88	0.140	0.040	0.67	0.32	0.26	
48	5 yrs.	Very fat	64.43	13.42	1.02	0.19	20.50	3.28	6.00	1.01	0.150	0.130	0.67	0.33	0.22	
Lean flesh of loin																
18	.....	.....	59.90	21.72	0.79	0.16	17.94	2.87	4.78	0.78	0.120	0.000	0.55	0.26	0.17	
121	.....	.....	67.22	11.40	0.94	0.19	18.81	3.01	5.40	0.87	0.130	0.040	0.65	0.31	0.26	
48	.....	.....	61.78	18.26	0.96	0.17	18.63	2.98	5.25	0.79	0.140	0.100	0.62	0.31	0.19	
Lean flesh of rib																
18	.....	.....	62.79	18.04	0.82	0.16	19.25	3.08	4.63	0.72	0.110	0.100	0.54	0.27	0.18	
121	.....	.....	60.98	20.87	0.78	0.15	17.44	2.79	4.55	0.71	0.110	0.040	0.55	0.25	0.21	
48	.....	.....	56.86	24.79	0.83	0.15	16.75	2.68	4.44	0.69	0.120	0.110	0.50	0.21	0.17	
Lean flesh																
594	11 mo.	Fat	71.31	6.88	0.90	0.19	20.50	3.28	5.00	0.90	0.148	0.145	0.62	0.28	0.22	
591	17 mo.	Very thin	74.24	2.78	0.93	0.19	20.25	3.24	5.18	0.84	0.153	0.088	0.63	0.28	0.23	
597	17 mo.	Medium fat	71.29	8.54	0.93	0.18	18.88	3.02	4.96	0.77	0.141	0.108	0.59	0.28	0.22	
593	17 mo.	Good	71.40	6.21	0.98	0.19	18.94	3.03	4.54	0.62	0.137	0.103	0.55	0.26	0.21	
595	23 mo.	Thin	73.52	4.91	1.00	0.20	19.81	3.17	4.98	0.77	0.153	0.135	0.56	0.26	0.22	
592	22 mo.	Very, very thin	76.37	1.87	1.05	0.17	18.94	3.03	4.21	0.80	0.128	0.033	0.40	0.15	0.20	
Cow 63	6 yrs.	Thin	68.70	9.52	0.98	0.19	20.00	3.20	5.17	0.78	0.145	0.125	0.60	0.25	0.21	
Cow 4	9½ yrs.	Fat	67.30	11.67	1.00	0.19	19.44	3.11	5.20	0.74	0.188	0.095	0.60	0.27	0.21	
Cow 43	7 yrs.	Fat	67.12	12.70	0.97	0.18	19.19	3.07	4.90	0.69	0.135	0.096	0.59	0.24	0.27	

enough for the precipitate to settle. The liquid is then filtered or decanted according to the nature of the precipitate. Six hundred c.c. of this are measured into 800 c.c. beakers and treated as under total soluble phosphorus. The last aliquot part of 600 c.c. represents five-sixths of the original sample, which is 10 grm. of lean meat or 15 grm. of fat meat.

**Soluble Inorganic Phosphorus.**—The difference between the total soluble phosphorus and the soluble organic phosphorus is considered to be inorganic phosphorus.

**Some Results of the Missouri Method.**—Ritchie, Moulton, Trowbridge, and Haigh (*Missouri Agr. Expt. Sta., Research Bull.* 59, 1923) have reported a large number of results of the application of the water-extract method to beef flesh. A few typical results are shown on p. 305.

**Some Results by Richardson's Method.**—The tables on p. 307 and 308 show the composition of fresh lean beef according to the general methods of analysis used by Richardson and Scherubel. In making these analyses it was thought advisable to select the leanest and most uniform muscular tissue which could be found. The part selected is commonly known as the beef "knuckle" among butchers, and is the crural triceps of anatomists, consisting of the rectus femoris, vastus externus, vastus internus, and anterior gracilis muscles.

### Hot-water Extract

It is sometimes desired to make a hot-water extract of the tissue or product. Usually the residue from the cold-water extraction is used and is extracted several times by boiling with water. If 100 grm. are used, 4 extractions of 1 hour each, using 250 c.c. of water each time, should be sufficient; but the process should be continued until practically no more gelatin is extracted, as shown by the biuret test. If the cold-water extraction has been carried out properly, the hot-water extract will consist chiefly of gelatin (or gelatone) and smaller quantities of peptones derived from insoluble or coagulable proteins. Each extract is strained through cheese-cloth (placed in a funnel) into a 100 c.c. flask. Some chemists combine the hot-water extract with the cold-water extract and analyse the whole at one time, but a better procedure is to analyse them separately. If the latter course be followed, it is usually sufficient

PART I.—ANALYSES OF FRESH BEEF KNUCKLES. ALL SAMPLES HELD AT 2-4° UNTIL ANALYSED. AGE, 0-7 DAYS.  
ALL FIGURES ON BASIS OF ORIGINAL MEAT

Source	Lab. No.	Killed	Ana-lysed days	Mois-ture %	Ash %	Pat. spt. %	Total N. %	Amm. N. Method 1 %	Amm. N. Method 2 %	Cold water extract				
										Total solids %	Ash %	Organic %	Total N. %	Coag. N. %
Unknown.....	.....	11/9	11/9	76.78	1.11	1.93	3.41	0.029	.....	6.24	0.97	5.27	0.807	0.444
Choice steer.....	.....	1907	1907	76.78	1.11	1.93	3.41	0.029	.....	6.24	0.97	5.27	0.807	0.444
.....	3813	4/17	4/22	74.78	1.26	3.34	3.47	0.030	0.010	6.03	1.24	4.79	0.777	0.397
Old Bull.....	.....	1908	1908	76.20	1.31	1.08	3.65	0.033	0.110	6.09	1.27	4.82	0.849	0.448
.....	3814	4/17	4/22	76.20	1.31	1.08	3.65	0.033	0.110	6.09	1.27	4.82	0.849	0.448
Heifer.....	.....	1908	1908	75.26	1.23	2.30	3.38	0.029	0.010	6.02	1.17	4.85	0.795	0.413
.....	3815	4/21	4/22	75.26	1.23	2.30	3.38	0.029	0.010	6.02	1.17	4.85	0.795	0.413
Old cow.....	.....	1908	1908	77.27	1.23	0.95	3.46	0.027	0.011	5.55	1.13	4.42	0.742	0.358
.....	3816	4/21	4/22	77.27	1.23	0.95	3.46	0.027	0.011	5.55	1.13	4.42	0.742	0.358
Unknown.....	.....	1908	1908	77.17	1.20	1.05	3.45	0.028	0.010	6.16	1.16	5.00	0.854	0.452
.....	459	1/14	1/18	77.17	1.20	1.05	3.45	0.028	0.010	6.16	1.16	5.00	0.854	0.452
Unknown.....	.....	1908	1908	76.96	1.27	0.85	3.46	0.030	0.010	6.15	1.15	5.00	0.840	0.445
.....	460	1/16	1/20	76.96	1.27	0.85	3.46	0.030	0.010	6.15	1.15	5.00	0.840	0.445
Unknown.....	.....	1908	1908	76.28	1.30	1.28	3.43	0.028	0.009	5.78	1.11	4.67	0.778	0.393
.....	.....	4/2	4/6	76.28	1.30	1.28	3.43	0.028	0.009	5.78	1.11	4.67	0.778	0.393
Unknown.....	.....	1908	1908	76.30	1.28	0.78	3.56	0.033	0.011	6.02	1.22	4.90	0.837	0.409
.....	3263	4/2	4/6	76.30	1.28	0.78	3.56	0.033	0.011	6.02	1.22	4.90	0.837	0.409
Unknown.....	.....	1908	1908	76.33	1.27	0.90	3.54	0.032	0.010	6.20	1.23	4.97	0.812	0.394
.....	3264	10/12	10/15	76.33	1.27	0.90	3.54	0.032	0.010	6.20	1.23	4.97	0.812	0.394
Unknown.....	.....	1907	1907	76.73	1.13	1.82	3.34	.....	.....	6.02	0.95	5.07	0.766	0.401
.....	.....	3/2	3/6	76.73	1.13	1.82	3.34	.....	.....	6.02	0.95	5.07	0.766	0.401
Unknown.....	.....	1908	1908	77.04	1.28	1.06	3.43	0.022	0.010	5.94	1.25	4.69	0.840	0.433
.....	3266	10/12	10/19	77.04	1.28	1.06	3.43	0.022	0.010	5.94	1.25	4.69	0.840	0.433
Unknown.....	.....	1907	1907	75.56	1.10	1.25	3.59	.....	.....	5.89	1.06	4.83	0.787	0.393
Maximum.....	.....	.....	.....	75.56	1.10	1.25	3.59	.....	.....	5.89	1.06	4.83	0.787	0.393
Minimum.....	.....	.....	.....	77.27	1.31	3.43	3.65	0.033	0.011	6.24	1.27	5.27	0.854	0.452
.....	.....	.....	.....	77.27	1.31	3.43	3.65	0.033	0.011	6.24	1.27	5.27	0.854	0.452
Average.....	.....	.....	.....	75.26	1.11	0.78	3.34	0.022	0.009	5.55	0.95	4.42	0.742	0.358
.....	.....	.....	.....	75.26	1.11	0.78	3.34	0.022	0.009	5.55	0.95	4.42	0.742	0.358
.....	.....	.....	.....	76.35	1.23	1.43	3.49	0.029	0.010	6.01	1.14	4.67	0.800	0.413
.....	.....	.....	.....	76.35	1.23	1.43	3.49	0.029	0.010	6.01	1.14	4.67	0.800	0.413

PART II.—ANALYSES OF FRESH BEEF KNUCKLES. ALL SAMPLES HELD AT 2-4° UNTIL ANALYSED. AGE, 0-7 DAYS. FIGURES CALCULATED TO MOISTURE, ASH, AND FAT-FREE BASIS

Source	Lab. No.	Killed	Analysed	Age, days	Total N. %	Amm. N. Method 1 %	Amm. N. Method 2 %	Organic extractives %	Cold water extract			
									Total N. %	Coag. N. %	Albumose N. %	Meat base N. %
Unknown.....	....	11/9 1907	11/9 1907	0.0	16.90	0.144	.....	26.12	3.99	2.20	0.129	1.65
Choice steer.....	3813	4/17 1908	4/22 1908	5.0	16.83	0.145	0.048	23.21	3.77	1.92	0.107	1.81
Old bull.....	3814	4/17 1908	4/22 1908	5.0	17.05	0.154	0.051	22.51	3.96	2.09	0.126	1.80
Heifer.....	3815	4/21 1908	4/22 1908	1.0	16.88	0.137	0.047	22.87	3.75	1.95	0.099	1.71
Old cow.....	3816	4/21 1908	4/22 1908	1.0	16.83	0.131	0.053	21.51	3.61	1.74	0.107	1.75
Unknown.....	459	1/14 1908	1/18 1908	4.0	16.76	0.136	0.049	24.30	1.15	2.20	0.146	1.86
Unknown.....	460	1/14 1908	1/18 1908	4.0	16.54	0.143	0.048	23.90	4.02	2.13	0.162	1.79
Unknown.....	.....	1/26 1908	1/29 1908	3.0	16.22	0.132	0.043	22.09	3.64	1.86	0.118	1.74
Unknown.....	3263	4/2 1908	4/6 1908	4.0	16.45	0.152	0.051	22.64	3.87	1.89	0.111	1.84
Unknown.....	3264	4/2 1908	4/6 1908	4.0	16.47	0.119	0.047	23.12	3.78	1.83	0.112	1.82
Unknown.....	.....	10/12 1907	10/15 1907	3.0	16.44	.....	.....	24.65	3.77	1.97	0.069	1.79
Unknown.....	2326	3/2 1908	3/9 1908	7.0	16.63	0.107	0.048	22.75	4.07	2.05	0.092	1.83
Unknown.....	.....	10/12 1907	10/19 1907	7.0	16.25	.....	.....	21.87	3.56	1.78	0.149	1.61
Maximum.....	.....	.....	.....	7.0	17.05	0.154	0.053	20.12	4.15	2.20	0.162	1.86
Minimum.....	.....	.....	.....	0.0	16.22	0.107	0.043	21.51	3.56	1.74	0.069	1.61
Average.....	.....	.....	.....	3.7	16.63	0.139	0.049	23.22	3.84	1.97	0.117	1.77

to estimate the nitrogen in an aliquot part of the hot-water extract, and calculate to collagen (or gelatin) by using the factor 5.55.

### Extraction with Solutions of Salts

Usually the foregoing methods are found adequate for the investigation in hand. But certain other methods are applicable to certain cases and are used more often in research work than in routine examinations. Solutions of neutral salts may be employed to extract soluble matter, especially the proteins. Sodium chloride is the salt commonly used, but other salts have been employed. Some of these methods will be described.

**Extraction with Sodium Chloride Solutions.**— That the extraction and the solubility of the nitrogenous substances in meat is not a simple matter, is indicated not only by the fact that water does not extract as much of the soluble substances as are present in expressed meat juice, but by the following figures (Richardson and Scherubel, *J. Amer. Chem. Soc.*, 1908, **30**, 1543) which show the amounts of solids and organic nitrogen dissolved from hashed beef by solutions of sodium chloride of different strengths. Such solutions might be used to separate nitrogenous substances in meat.

#### EXTRACTION OF BEEF KNUCKLES WITH WATER AND SOLUTIONS OF SALTS OF DIFFERENT CONCENTRATIONS

100 gm. meat extracted with the solvent and extract made up to 1 litre; 50 c.c. (representing 5 gm. of meat) taken for each estimation. Two experiments (a) and (b).

Solvent	Solids in 50 c.c. solution gm.	Ash in 50 c.c. solution gm.	Meat solids in solution %	Organic extra- ctives %	Meat ash %	Total nitrogen %
Distilled water	(a) 0.2824 (b) 0.2625	(a) 0.0597 (b) 0.0540	(a) 5.65 (b) 5.25	(a) 4.46 (b) 4.17	(a) 1.19 (b) 1.08	(a) 0.770 (b) 0.651
0.6% salt	(a) 0.6097 (b) 0.6412	(a) 0.3441 (b) 0.3227	(a) 6.50 (b) 6.45	(a) 5.31 (b) 5.37	(a) 1.19 (b) 1.08	(a) 0.931 (b) 0.858
2.0% salt	(a) 1.2688 (b) 1.2075	(a) 0.9580 (b) . . . . .	(a) 7.40 (b) . . . . .	(a) 6.21 (b) . . . . .	(a) 1.19 (b) 1.08	(a) 1.080 (b) 0.913
5.0% salt	(a) 2.6200 (b) 2.5252	(a) 2.2392 (b) 2.1517	(a) 8.81 (b) 8.55	(a) 7.62 (b) 7.47	(a) 1.19 (b) 1.08	(a) 1.232 (b) 1.158
10.0% salt	(a) 4.8024 (b) 4.7807	(a) 4.4465 (b) 4.4216	(a) 8.30 (b) 8.38	(a) 7.11 (b) 7.30	(a) 1.19 (b) 1.08	(a) 1.322 (b) 1.274
20.0% salt	(a) 9.1873 (b) . . . . .	(a) 8.8395 (b) . . . . .	(a) 8.14 (b) . . . . .	(a) 6.95 (b) . . . . .	(a) 1.19 (b) . . . . .	(a) 1.001 (b) . . . . .
1.1% K <sub>2</sub> HPO <sub>4</sub>	(a) 0.8388 (b) 0.8194	(a) 0.5145 (b) . . . . .	(a) 7.67 (b) . . . . .	(a) 6.48 (b) . . . . .	(a) 1.19 (b) . . . . .	(a) 0.806 (b) 0.858

The 10% salt solution appears to extract the largest amount of nitrogenous substances, possibly not without alteration, whereas



the 5% solution extracts more organic solids. However, on account of the large quantity of salt present, the ash figures are not to be considered as being absolutely accurate, although the estimations were made as carefully as possible. The nitrogen figures should be considered more accurate. In the case of the 5 and 10% salt solutions, results in soluble meat solids and nitrogen are obtained of the same order as those representing the soluble solids and nitrogen in meat juice itself, calculated to the basis of the meat.

**Extraction with Potassium Phosphate.**—In addition to sodium chloride, other salts have been used to extract the nitrogenous substances of flesh. Among these, ammonium sulphate has been prominent. Grindley used it extensively in his work after the cold-water extract. Howe (*J. Biol. Chem.*, 1924, **61**, 493) has employed various salts, such as sodium sulphate, lithium sulphate, magnesium sulphate, sodium phosphate and potassium phosphate, in addition to the two named just above. He employed different molar concentrations and successive extractions. He obtained the maximum extraction of proteins with potassium phosphate solutions between 0.225 and 0.525 molar. For this purpose he used a mixture of 1 part of potassium dihydrogen phosphate to 2 parts of dipotassium hydrogen phosphate. Howe found that the solubility was affected by the acidity of the salt solution. Maximum extraction was obtained at pH 7, lower concentrations of hydrogen ions extracting less, and higher concentrations extracting no more. The ratio of phosphates used gave a pH of 7. When thus extracted, calf muscle gave from 1.4 to 1.5 gm. of soluble nitrogen per 100 gm. of flesh, and rabbit muscle gave 1.8 to 1.9 gm. The soluble nitrogen was thus about 50 per cent of the total nitrogen.

The muscles (not perfused) were removed from the animal as soon as possible after death—about one half to one hour between death and the addition of the salt solution. The meat was ground in a meat grinder and 5 gm. were weighed into a given quantity of the salt solution of such a concentration that the water of the muscle would give the desired final concentration of salt, assuming that there was 4 c.c. of water in the 5 gm. of muscle.

The following are some results obtained by this method. The figures in some instances are slightly different from Howe's, since the author has recalculated the average figures shown.

DISTRIBUTION OF NITROGEN IN MUSCLE—(HOWE)  
Results expressed in percentage of fresh muscle

	Total nitro- gen	Total solu- ble nitro- gen	Myo- sin nitro- gen	Myo- gen nitro- gen	Total glob- ulin nitro- gen	Albu- min nitro- gen	Non- pro- tein nitro- gen	Insol- uble nitro- gen
Calf								
Weight per 100 grm. flesh . . .	3.07	1.42	0.49	0.42	0.91	0.18	0.34	1.65
Per cent of total nitrogen . . .	46.3	16.0	13.7	29.7	5.5	11.1	53.7	
Cow and Bull								
Weight per 100 grm. flesh . . .	2.95	1.69	0.67	0.43	1.10	0.18	0.41	1.26
Per cent of total nitrogen . . .	57.3	22.7	14.6	37.3	6.1	13.9	42.7	
Rabbit								
Weight per 100 grm. flesh . . .	3.57	1.79	0.72	0.51	1.20	0.15	0.44	1.78
Per cent of total nitrogen . . .	50.1	20.2	14.3	33.6	4.2	12.3	49.9	

Howe prefers the nomenclature of Halliburton and uses the terms paramyosinogen and myosinogen where the author has used myosin and myogen. Howe also classes both proteins as globulins. This does not appear to be justifiable. The distribution of the nitrogen between the two proteins is vastly different from that reported by Mathews (see p. 254) who states that the albumin is 3 to 4 times as great in quantity as the globulin. Howe's figures show that the globulin nitrogen as extracted and determined by him, is 6 to 8 times the albumin.

**Other Methods of Salt Extraction.**—Ritchie and Hogan (*J. Amer. Chem. Soc.*, 1929, **51**, 880) report some results of their investigation of the proteins of rabbit muscle. The flesh was perfused with Ringer-Locke solution until this came free from colour. The flesh was then frozen with carbon dioxide and ground in a mortar. Other samples were ground in a Nixtamal mill, which gave a very finely divided sample. The latter method is recommended. Samples of 100–125 grm. were placed in 200 c.c. bottles and 100 c.c. of the extracting liquid was added. The bottles and mixture were shaken gently and allowed to stand 1 hour. The bottles were then centrifuged and the supernatant liquid was poured off. The extraction was then repeated. Finally, the liquid was made up to 2 litres for 100 grm. samples, and 2.5 litres for 125 grm. samples. The liquid was filtered through cellucotton on a Büchner funnel. All samples and solutions were kept packed in a mixture of ice and salt. The extracting fluid was 10% sodium chloride adjusted to a pH of about

6 (5.9–6.1). The globulin was precipitated by saturating with sodium chloride, by dialysis, by exposure to irradiation by a quartz mercury arc, or by precipitation at 49°. The albumin, or the globulin and albumin together, were precipitated by heating to boiling and adding trichloroacetic acid to a final concentration of 2.5%. A typical result is shown.

DISTRIBUTION OF SOLUBLE NITROGEN OF RABBIT FLESH

	Saturation %	Dialysis %	Irradiation %	Heat and acid %
Globulin.....	61.4	53.8	43.5	84.5
Albumin.....	27.1	29.9	22.0	
Non-protein.....	18.8	21.8	8.5	

A different sample of extract coagulated at 45° gave globulin 57.6%, albumin 33.3%, non-protein nitrogen 9.8%.

These results of Ritchie and Hogan show 2.5 times as much globulin as albumin nitrogen. This result lies between those of Howe and the figures given by Mathews. It should be remembered that authorities state that the precipitation limits with ammonium sulphate should not be used to distinguish globulins from albumins. However, it has been found convenient to precipitate globulins from solution by half saturation with ammonium sulphate or full saturation with sodium chloride. In the opinion of the author, the terms globulin and albumin, as applied to flesh, will mean little so long as a change in method gives such variations in the relations of these two proteins.

Hoagland, McBryde, and Powick (*U. S. Dept. Agr., Bull.* 433, 1917, 12) have preferred to use a salt solution of lower concentration in their examination of flesh. They used a 0.9% aqueous solution of sodium chloride saturated with thymol in their investigation of the changes that occur in fresh beef during cold storage. Taking all the facts concerning the nature of the constituents of muscular tissue into consideration, they concluded that the use of a solvent is tonic with the muscle serum would throw the most light upon the nature and quantity of the soluble constituents of the muscles, either in fresh condition or after autolysis or cold storage. By their method fresh beef, 72 hours in a cooler after slaughter, gave about 1% of soluble nitrogen out of a total of about 3.3%. The soluble nitrogen was divided between coagulable and non-coagulable

not quite evenly, the figures being 0.51% of coagulable and 0.44% of non-coagulable. No attempt was made to separate globulin and albumin.

### Alcohol Separation of Nitrogenous Substances

The alcoholic separation of nitrogenous substances (see also pages 508, 514) has been applied by Knorr (*U. S. Agric. Report*, 1886, 355-357) to the extraction of the meat bases from the meat. His method is as follows: A suitable quantity of the fat-free sample is extracted with strong alcohol, which dissolves the meat bases, together with small amounts of non-nitrogenous extractives such as lactic acid, glycogen, and inositol. The alcohol extract is dried and weighed and the nitrogen determined in the residue, as usual. A cold-water extract is made of another portion—removing certain soluble proteins and the meat bases—and an aliquot part of the extract is dried and weighed, and the nitrogen therein determined. In another aliquot portion of the cold-water extract, the coagulable proteins are determined by heating the solution to boiling, filtering off the precipitate, drying it at 100°, and weighing it.

Knorr's average results from 11 analyses of meat are shown in the following table:

	%
Dried residue from cold-water extract.....	13.76
Dried coagulable albumin . . . . .	2.24
Total cold-water extract.. . . .	3.56
Total nitrogen . . . . .	3.37
Nitrogen in residue from alcohol extraction . . . . .	2.86
Dried alcohol extract.....	3.03

### Other Methods

**Mallet's phosphotungstic acid method and the bromine precipitation method** (*U. S. Dept. Agr., Bur. Chem., Bull.* 54) (see p. 516) have been applied to the separation of nitrogenous substances, but are not so useful as the more recent methods described. In both cases the separations are not sharp, and the results do not usually agree as well as could be desired.

## THE COOKING OF MEAT

Meat is tender before the occurrence of *rigor mortis*, but when once that condition has set in, it requires to be kept some days before it again acquires this character.

The tangible change in composition undergone by meat in the process of roasting consists chiefly in the loss of water by evaporation and formation of gravy, with the loss of fat in the form of dripping. The proteins become concentrated in the cooked meat, while at the same time certain dark-coloured substances of carameloid nature are formed, which greatly modify and improve the flavour and odour of the meat.

When meat is boiled in water, a considerable quantity of organic and inorganic matters pass into solution, and when the liquid is not intended to be consumed, the loss should be reduced to a minimum by immersing the meat in boiling water for a few minutes, and then adding more water, in quantity sufficient to reduce the temperature of the liquid to about  $77^{\circ}$ , which temperature should not be greatly exceeded during the remainder of the process of cooking. By operating in this manner an insoluble coating of coagulated proteins is formed on the meat, and loss by solution is reduced to a minimum. On the contrary, when it is desired to extract the meat as thoroughly as possible, as in preparing soup, beef-tea, or mutton-broth, the meat should be placed in cold water, and the temperature gradually raised.

**Soup** contains the extractives of the meat from which it is prepared, a portion of the proteins, and most of the gelatinoids.

**Beef-tea** contains only insignificant quantities of proteins, gelatinoids or fat, and hence possesses true nutritive properties to but a very limited extent. Its value appears to be due to the stimulating action of the extractives, especially creatine, creatinine, xanthine, lactic acid, and salts.

**Liebig's extract of meat** is practically concentrated beef-tea, and owes its value to the same constituents. The nature of commercial meat extracts is discussed fully later (see p. 464).

The effects of cooking on meat have been very fully investigated and discussed by Grindley and his associates (*U. S. Dept. Agric. Off. Exp. Sta. Bulls.* 102, 141, 162, and 193, 1901-1907). The principal points of interest for the analyst in connection with the cooking of meat are the coagulation and hydrolysis of the proteins and the great shrinkage in weight, due chiefly to the loss of water coincident with the coagulation of the proteins. Cooked meats usually contain 55-65% water, whereas fresh meats contain 70-75%. The shrinkage in weight usually amounts to 30-40% of the original

weight. The lower the temperature and the shorter the time of cooking, the less the shrinkage. Cooking commences when the proteins of lowest coagulation point begin to coagulate, but the temperatures generally employed vary from 65° to a little above 100°, or to a considerably higher temperature on the surface only. Grindley (*loc. cit.*) concludes that when beef is cooked in water, from 3 to 20% of the total solids is found in the broth, but that beef which has been used for the preparation of beef-tea or broth has lost comparatively little of its nutritive value, though much of the flavouring material has been removed.

### CHANGES IN FLESH FOLLOWING DEATH

Alteration occurs in flesh foods in 3 ways: (1) physically; (2) chemically; (3) biochemically.

The physical changes are those which affect the appearance, the structure of the tissue, macroscopically and microscopically, and, in general, changes noticeable by the senses, which do not affect the composition or nutritive value of the food.

The chemical and biochemical changes are those which affect the composition of the food, hence the nutritive value (advantageously or otherwise), and which may produce alterations in odour and flavour. The chemical agents of deterioration in the case of flesh foods are water and oxygen; in the absence of the former—that is, in desiccated flesh foods—the changes which occur in the lean portions, even after long storage at ordinary temperatures, are small. In the absence of water and oxygen—for instance desiccated meat *in vacuo*—flesh foods will keep indefinitely without change. In desiccated flesh foods, if oxygen is present, rancidity usually occurs after a shorter or longer period of storage; this change is entirely chemical in nature and does not require the presence of microorganisms. Practically all the spoilage which occurs in normal flesh foods is due to bacteria or moulds; that is, these micro-organisms are the exciting causes of the changes which occur, and in their absence, even though water and oxygen are abundantly present, the changes will be slow.

A tabular view of deterioration in the fat, as well as in the lean of meat, may be presented in the following form. In this scheme the formation of bacterial toxins is not considered, and this topic will be discussed later.

## CHEMICAL AND BIOCHEMICAL DETERIORATION OF FLESH FOODS

Three principal causes of influences	Fat	Hydrolysis—active agent water—products fatty acids and glycerol.
		Oxidation (rancidity) active agents water and oxygen; products lower fatty acids, aldehydes, etc.
Chemical (1) Water (2) Oxygen of air		Hydrolysis
		Oxidation
Biological (3) Micro-organisms	Lean	Decomposition by micro-organisms. Hydrolysis chief chemical action. In presence of oxygen oxidation plays a part, the sulphur compounds being affected and much CO <sub>2</sub> being evolved.
		Putrefaction (anaerobic)
		Decay (aerobic)
		Mouldering (aerobic) by moulds

In the absence of micro-organisms this occurs slowly at ordinary temperatures; rapidly at high temperatures, with production of gelatin and albumoses (proteoses). At ordinary temperatures enzymes act as accelerating agents. Occurs slowly at ordinary and moderately high temperatures, in presence of oxygen of air, even in absence of micro-organisms. Putrefaction produces reduction products of foul odour (NH<sub>3</sub>, H<sub>2</sub>S, amines, skatole (acids). Decay produces products with scarcely any pronounced disagreeable odour (no H<sub>2</sub>S). Mouldering produces the typical odour of "mouldiness," possibly due to acid amides

In the living animal, muscular tissue consists chiefly of semi-fluid muscle plasma, which has a faintly alkaline reaction. After the death of the animal, *rigor mortis* sets in, the meat becomes acid in reaction, due to the formation of lactic acid, and the previously soluble proteins coagulate in part to form the muscle clot, the principal proteins of which are myosin and myogen.

Moran and Smith (*Dept. of Scientific and Ind. Research, Food Investigation, Special Report No. 36*, London, 1929) describe *rigor mortis* as a pronounced hardening and shortening of the muscles, so that a carcass becomes rigid and the joints stiff. Meat in this condition is said to have "set." In beef the process is normally complete in about 24 hours. To quote from their work:

"If the process is studied on a normal intact muscle, the following changes are constantly observed: (a) hardening and stiffening; (b) shortening; (c) loss of elasticity; (d) loss of transparency; (e) loss of power to contract when electrically stimulated; (f) formation of acid (lactic acid); and (g) production of heat (heat of rigor).

"The rate of development of all these changes appears to follow the rate of increase in acidity, so that the hypothesis has been formed that rigor is actually caused by the action of lactic acid on the muscle-substance, causing this to coagulate in the same way that acid will cause the syrupy liquid expressed from living muscle to coagulate. This view is supported by the similar state into which muscle is

brought by heating above 45° C. heat-rigor, which is undoubtedly due to coagulation of the muscle-substance.

"It has recently been shown, however, (Hoet and Marks, *Proc. Roy. Soc.*, 1926, **100B**, 72) that the formation of lactic acid is not a necessary accompaniment of rigor. In animals which have had their reserves of glycogen (animal-starch) depleted, the onset of rigor is almost immediate after death, and is of short duration. (Normally, after attaining its maximum, the stiffness of the muscle gradually diminishes, so that after some days the joints again become loose and the muscles flaccid. This process is known as the resolution of rigor.) The muscle does not shorten, nor become opaque, and no increase in acidity takes place."

Expressed muscle substance, according to Kühne (*Untersuchungen über das Protoplasma*, W. Engelmann, Leipzig, 1864), who first examined this muscle plasma under rigidly controlled conditions, is usually alkaline on expression, but becomes acid after some time at room temperature, and a jelly is formed. Gelation may occur without the production of acid, and in this case the clot is transparent. However, if acid is produced later, the clot becomes opaque and contracts, exuding a thin, clear serum. Thus the formation of jelly appears to be independent of the production of acid. There are two stages in the process which may be distinguished. The first is the formation of a clear jelly without change in acidity, and the second is the action of acid on this jelly, causing contraction and opalescence.

Moran and Smith observe that these two stages correspond very closely to the events observed in the two types of rigor and the conclusion may be drawn that *rigor mortis* consists of the following two processes occurring simultaneously: (1) gelation of the muscle plasma with hardening and loss of response; and (2) acid production resulting in contraction of the muscle, increase in opacity, and coagulation and breakdown of the protein gel a bit later. An acid serum then exudes.

Moran and Smith have measured the heat of rigor of beef muscle and found it to be 1.2° C. (2.2° F.), which is reached in about 2½ hours after death. They discuss the cause of this rise and show that, while the glycogen present can account for the rise in temperature by its change to lactic acid, other considerations show that this is not the whole explanation. Such considerations are that rigor is



not complete until at least 24 hours after death, that the breakdown of glycogen and formation of lactic acid are not complete in 2 hours, and that, at least in rats, the heat of rigor is of a much higher order. Experimental results are reported which show that at 37° C. between 0.6 and 0.7% of lactic acid is formed in about 6 hours in beef, while at 21° C. about 23 hours are required.

Earlier investigators looked to the muscle carbohydrates as the source of lactic acid, but later investigations indicate that it may have a protein origin. The onset of *rigor mortis* may be delayed in various ways, as by low temperatures or an abundant supply of oxygen; but in any event it gradually passes away and the muscular tissue again relaxes. This relaxation is coincident with the process known as ripening which is in effect an auto-digestion which causes the meat to become more tender. The ripening process is due to proteoclastic enzyme (protase) naturally present in meat and is not caused by bacterial decomposition, although bacterial decomposition may overtake it and become the dominant effect when meat is held too long or at too high a temperature or in too small pieces. A slight bacterial decomposition, or "gamey" flavour, is desired by some persons, and meat of this sort is eaten by them with no apparent ill effects. The ripening process in the case of large pieces (sides or quarters of beef) held at 34 to 38° F. reaches its maximum in from 15 to 21 days, when bacterial action has not penetrated much below the surface of the meat (about  $\frac{1}{16}$  to  $\frac{1}{8}$  in.), but beef is sometimes held at these temperatures by hotels, etc., for 30 and even 60 days.

Moran and Smith, in discussing the changes following rigor, state that these changes are relatively slow. The muscle still contains a group of active enzymes, and these, together with the high concentration of lactic acid produced during rigor, are responsible for a gradual breakdown of the substance of the fibres. The muscle again becomes soft and pliable, and, if the meat is stored for still longer periods, the proteins themselves are gradually hydrolysed into simpler bodies.

The rate at which these changes take place depends upon the temperature, higher temperatures speeding up the action, while the change continues slowly at a temperature above the freezing point. During storage below the freezing point the changes are almost completely inhibited. Thus frozen meat properly stored

keeps well, excepting for drying out, for long periods of time, while meat may be ripened at a temperature near the freezing point for shorter lengths of time. In commercial practice this ripening is limited to from 2 to 6 weeks.

In the case of normal storage above the freezing point both bacteria and moulds have to be considered. Meat, as well as other foods containing much water and food for bacteria, is a good host for the growth of micro-organisms, and they take a part in further changes occurring in the muscle. The practice of chilling, storing and handling meat is largely aimed at the prevention or control of growth of these organisms.

The bacteria which cause the decomposition of flesh foods gain access to the meat largely after the death of the animal. It has been, and still may be, the consensus of scientific opinion that normal muscular tissue from an animal healthy at the time of slaughter is free from bacteria at that time. It is recognised that bacteria sometimes gain access to the circulation during life, for example, during agonal periods. The blood promptly kills them during health or otherwise puts them out of action.

A new point of view concerning the presence of bacteria in flesh has been presented by Reith (*J. Amer. Med. Assoc.*, 1926, **136**, 325; *J. Bacter.*, 1926, **12**, 367). Employing a technique carefully worked out under Jordan and Norton at the University of Chicago, Reith has demonstrated the presence of both aerobes and anaerobes in the flesh of live hogs, guinea pigs and rabbits, and even in the blood stream. Not all preparations showed the presence of bacteria.

These results are so at variance with the usual point of view that one may, perhaps, be pardoned for looking upon them critically. Support is given them, however, by the work of Hoagland, McBryde, and Powick (*U. S. Dept. Agr., Bull.* **433**, 1917) who carried out autolysis experiments with beef. They employed a well-worked out technique to obtain meat under aseptic conditions, but were able to secure only 12 samples out of 36 showing no bacterial growth on incubation. Contamination may be blamed for the failure in 24 cases, but there still remains the possibility of the flesh itself carrying bacteria.

Whichever point of view is correct, the practical result is the same. Packing-house practice will still aim at sanitation and prevention of contamination, for it is of importance to keep down

the extent of the contamination, and its main reliance will be upon prompt and effective chilling of the carcass, maintenance of low temperatures, adequate curing, and sterilisation in the case of regular canned meats.

The author has discussed this question at some length elsewhere (*Meat through the Microscope*, University of Chicago Press, Chicago, 1929, p. 123). Both McBryde (*U. S. Dept. Agr., Bur. Animal Ind., Bull.* **132**, 1911) and Boyer (*Amer. Food J.*, 1923, **18**, 197; *J. Agr. Research*, 1926, **33**, 761) have studied the question of the spoilage of meat.

**The Spoilage of Meat.**—If adequate precautions are not taken to ensure its preservation, meat undergoes progressive decomposition, beginning with the death of the animal from which it was derived. This process can best be followed in the case of large pieces of beef held in chill rooms at 1 to 3° (34 to 38° F.), which is the common modern practice. Into these rooms the sides are placed immediately after killing and dressing, and they are held there until transferred to the refrigerator car or ship for shipment to the consumer or middleman.

Starting with recently slaughtered meat free from bacteria, there are two ways by which micro-organisms enter the flesh and excite decomposition: first, by direct extension of surface colonies into the firm tissue; secondly, by extension and locomotion along surfaces such as those afforded by blood- and lymph-vessels, and nerve and connective-tissue sheaths.

By the first method, penetration is slow and very regular. The progress of the extension of the innumerable bacteria from the surface toward the interior can be easily observed. If a section be cut across a lean piece of beef which has been stored in the cooler for about 20 days or more, a dark, narrow, sharply defined border can be seen extending all around the cut. The line separating this border from the interior marks the limit of bacterial penetration by the first method. It will readily be seen that motility cannot play a great part in this process; rather the advance occurs by the actual extension of the limits of those confluent colonies which have spread over the surface and later penetrated the meat. It will also be evident that the harder and drier the surface the less readily will penetration occur at the start, although after the surface layer is pierced, progress will be easier. Hence the advantage of a certain

amount of desiccation in the cooler in the preservation of meats. Again, the connective tissue which surrounds muscles, and with which the ordinary large cuts of beef, mutton, and pork are more or less covered, is especially difficult of penetration by bacteria when it is dry. In this condition it resembles parchment.

The width of the bacterial border shown in section increases with the time of storage. Under favourable conditions at 2° to 4° it will be about 1 cm. in 30 days; under unfavourable conditions (good desiccation) it may be only 2 or 3 mm. in the same length of time. Fresh beef formerly shipped abroad from Chicago reached the ports of England 3 or 4 weeks after the death of the animal. It is needless to say that it arrived in a satisfactory condition of preservation.

It is characteristic of this method of bacterial penetration that the line of demarcation between the decomposing surface and undecomposed interior remains always sharp and distinct.

The second method of penetration, wherein bacteria follow various moist surfaces and tubes, including arteries, veins, nerve sheaths, lymph-vessels, and connective tissue, allows them to reach at a relatively early period the interior of the meat, where they set up foci of decomposition which spread and finally merge with each other and with the surface decomposition until the meat contains bacteria throughout and is in an active state of putrefaction. The extension of the interior foci can be roughly followed by making smear or impression preparations from various places on the surface of the sectioned meat, on cover-glasses or slides. In the earlier stages of decomposition, infected and uninfected areas will be found side by side, the infected spots will be few and small, the untainted areas large. Gradually the former increase in number and size; the latter diminish until with the encroachment of the surface layer of bacteria no uncontaminated spot remains.

So far as the external surface is concerned, if the air is dry, no slime will form. If the air (and hence the surface of the meat) is moist, a thick layer of slime will form, and later an abundant growth of mould will appear. The slime consists of quantities of bacteria. The ordinary bacterial decomposition of meat—except on the external surface— is a typical putrefaction.

In this decomposition the tendency is chiefly towards the formation of simpler compounds by hydrolysis, and if the various changes are able to reach the limit, on account of a sufficiently abundant

supply of water (which, among other functions, serves to dilute and render ineffective inhibitory substances) and of oxygen, the final products will be principally three: namely, water, ammonia, and carbon dioxide. The sulphur compounds will remain as sulphates, and the phosphorus compounds as phosphates. While the tendency of bacterial decomposition of flesh is principally in the direction indicated, at the same time the bacterial cells produce certain complex substances synthetically out of simpler materials.

Again, the decomposition process does not follow a uniform course from complex to simplest compounds, but even in the beginning ammonia, hydrogen sulphide, and other simple compounds of pronounced odour are split off and afford a basis for the chemical detection of decomposition.

### Methods for the Detection of Decomposition in Meat

**By Inspection.**—If the sample under investigation has not been specially treated in any way, but is simply chilled meat held at temperatures above the freezing-point, organoleptic tests and especially the odour afford one of the best means of judging unsoundness. Taste is of value in many instances and appearance in some, but the last is at times particularly misleading, although it may be a guide as to the previous handling of the meat. Experience is, of course, the first requisite in forming a judgment. An expert can distinguish slight different characteristic odours in the flesh of different animals, but good meat from any source has no pronounced odour, and especially no pronounced disagreeable odour, whereas in decomposing meat a strong “musty,” “mouldy,” “sour,” or “putrefactive” odour can be detected. The last is due largely to ammonia and hydrogen sulphide.

**By Microscopic Examination.**—It is very doubtful whether histological examinations afford any reliable data for the detection of decomposition in meat especially in the earlier stages. Wiley and Pennington (*Cold Storage Results on Fowl and Eggs*. Abst. Science, 1908, 27, 295; Bull. 115, Bur. Chem. U. S. Dept. Agr., 1908) report progressive deterioration in frozen meats on histological examination, but Richardson and Scherubel (*J. Amer. Chem. Soc.*, 1908, 30, 1515-64) have shown that the changes reported are probably physical effects due to freezing. Although histological examination is of little value in detecting decomposition, microscopic

examination for bacteria will always discover them in large numbers in decomposing meat. The only likely error in this examination is failure to locate the seat of decomposition. Smear preparations should be made at several points, stained with methylene blue, and examined with oil-immersion objective as usual.

**By Testing for Hydrogen Sulphide.**—*Eber's Sulphide Test* (*Z. Fleisch. Milch. Hyg.*, 1897, 7, 207, 227; 8, 41). In the bacterial decomposition of meat, among the first products to be split off from the proteins is hydrogen sulphide, and consequently when proper precautions are observed, a method for the detection of unsound meat can be based upon this fact. Eber applied his test to meat from diseased animals, especially tuberculous animals, and found that, in general, such meats yielded more hydrogen sulphide than meats from healthy animals. Fresh meat, when warmed on the water-bath, gives off hydrogen sulphide (and possibly other volatile sulphur compounds), but Eber found the quantities evolved in the case of healthy and diseased meats too nearly alike to distinguish them by this means. Working with the amounts evolved with dilute sulphuric acid, he obtained more characteristic results. His method is as follows: 25 grm. of the finely hashed sample are placed in an Erlenmeyer flask of convenient size, and 50 grm. of 10% sulphuric acid added. In the neck of the flask a strip of filter paper moistened with 10% lead acetate solution is fixed by means of a plug of cotton wool, and the apparatus is placed in a well-ventilated chamber in the dark for 24 hours at a temperature of about 15° (20–21° would be more convenient). At the end of the period the filter paper is compared with a standard set of similar strips. The colour will vary from yellowish-brown to black.

The set of standards can be made by treating a solution of pure potassium or sodium sulphide in the same way, using amounts varying from 0.002 mg. to 0.01 mg.

It is doubtful whether the details given above are the best which can be devised, and more work should be done with the method. As it is entirely empirical and comparative, in the absence of a standard method, each operator can work out details for his own conditions. It is a useful method in many cases. Meats which have been treated or cured with nitrates do not respond to Eber's sulphide test.

**By Estimation of Ammoniacal Nitrogen.**—The various methods for estimating the nitrogen in ammonium salts together with other

substances easily decomposed by means of weak alkalies, probably afford the best available chemical methods at the present time for the detection of decomposition in flesh foods. Various details for making the estimation have been proposed; but they all agree in treating the substance with a very weak alkali, volatilising the ammonia set free, absorbing it in standard acid, and titrating the excess. Although putrefactive processes occasionally give rise to acid products in excess of basic ones, as a general rule free ammonia is produced from first to last. Much ammonia is also left combined with various organic acids. Based on the free ammonia evolved from putrefying meat, Eber (*Arch. Wissensch. u. prak. Thierheilk.*, 1892, 18, 111; 1893, 19, 81) has proposed a qualitative test for incipient putrefaction as follows: the sides of a glass cylinder are moistened with a mixture of hydrochloric acid 1 part; alcohol 3 parts; and ether 1 part. A piece of the sample on the end of a wire is introduced into the cylinder, and if a white cloud of ammonium chloride forms, it is an indication of putrefaction.

The test might be modified to include those cases where acid products of putrefaction are in excess, by treating the hashed sample in an Erlenmeyer flask with barium carbonate at ordinary temperature and suspending a strip of filter paper moistened with the test solution mentioned above.

In estimating ammoniacal nitrogen quantitatively several difficulties arise. The aim of the estimation is to effect a separation of the simplest basic nitrogenous compounds—resulting from decomposition—from the more complex, but proteins themselves are decomposed by boiling with even the weakest alkalies and water and even by prolonged digestion with water itself. To avoid this result as far as possible three distinct principles are made use of:

(1) Use of the weakest alkalies as magnesium oxide and barium carbonate.

(2) Use of alcohol to reduce hydrolysis.

(3) Use of low temperatures, evolving the ammonia by air-aspiration.

(1) One hundred grm. of the well-hashed sample are weighed into a 1 litre round-bottomed distilling flask, and 10 grm. of freshly calcined magnesium oxide and 450 c.c. of water are added, and the whole mixed. The mixture is quickly brought to the boiling-point and the ammonia distilled into *N*/10 sulphuric acid, 50-60 minutes

being used in the distillation and exactly 200 c.c. being distilled over. Care is necessary in the distillation, especially at the start, to prevent sticking of the material to the bottom of the flask and charring. Cochineal indicator is used in the back titration. Comparable results are obtained in one distillation, but if sufficient water is added to replace the volume distilled and the distillation repeated, more ammonia comes over. After 4 or 5 distillations a practical limit is reached. The amount of ammoniacal nitrogen (calculated as nitrogen) found by this method in the lean meat from beef, pork, and chickens, lies between the limits 0.025% and 0.035%. (See tables below.)

(2) One hundred grm. of the sample are extracted with 150 c.c. of 60% (by volume) alcohol 3 times, the extract filtered through fine linen or good cheese-cloth, and the residue brought upon the filter cloth and wrung out tightly each time. To the combined extracts in a 1 litre round-bottomed distilling flask are added 10 grm. of recently calcined magnesium oxide, and exactly 200 c.c. are distilled into  $N/10$  sulphuric acid,  $\frac{1}{2}$  hour being taken for the distillation. Cochineal indicator is used in the back titration. A blank is made on reagents and solvent. Results by this method are, for fresh meats, about one-third to one-half those obtained by method 1.

Results by the two methods given above are shown in the following tables:

*Ammoniacal nitrogen obtained by successive distillations of hashed fresh meat, the contents of the flask being made up to volume between distillations.*

No. of distillation	Method 1		Method 2	
	%	%	%	%
1	0.030	0.029	0.010	0.010
2	0.015	0.014	0.001	0.001
3	0.000	0.000	0.001	0.001
4	0.006	0.007	0.001	0.001
5	0.005	0.005		
6	0.006	0.007		
7	0.005	0.005		
8	0.004	0.004		
9	0.004	0.004		
10	0.004	0.003		
Sum	0.088	0.087	0.013	0.013



*Ammoniacal nitrogen obtained by successive distillations of hashed frozen meat, the contents of the distillation flask being made up to volume between distillations.*

No. of distillation	Sample 1				Sample 2			
	Method 1 %		Method 2 %		Method 1 %		Method 2 %	
1	0.034	0.032	0.009	0.009	0.034	0.032	0.008	0.008
2	0.014	0.014	0.001	0.001	0.012	0.012	0.001	0.001
3	0.009	0.010	0.001	0.001	0.009	0.010	0.001	0.001
4	0.009	0.009	0.001	0.001	0.008	0.008	0.001	0.001
5	0.008	0.008	0.001	0.001	0.006	0.008	0.001	0.001
6	0.006	0.006	.....	.....	0.006	0.006	.....	.....
7	0.005	0.005	.....	.....	0.005	0.005	.....	.....
8	0.004	0.004	.....	.....	0.005	0.004	.....	.....
9	0.004	0.003	.....	.....	0.003	0.003	.....	.....
10	0.003	0.002	.....	.....	0.002	0.002	.....	.....
Sum	0.096	0.093	0.013	0.013	0.090	0.090	0.012	0.012

The following figures show the rise in ammoniacal nitrogen during putrefaction, the results being obtained by the methods given above.

*Putrefaction of Beef at Room Temperature (Summer) 24-33°.*

Twenty grm. meat digested with 450 c.c. of water after addition of 1 c.c. of putrefying infusion of meat.

Hours.....	0	5	21	29	45	93	117	165	309
Ammoniacal nitrogen method 1, %	0.038	0.039	0.069	0.099	0.182	0.621	1.056	1.379	1.648

*Experiment on Putrefaction of Beef at Room Temperature 24-33°.*

Twenty grm. meat digested with 100 c.c. of water after addition of 1 c.c. of putrefying meat infusion. Just before distillation enough alcohol was added to make 450 c.c. (total of 60% alcohol, Method 2).

Hours.....	0	16	64	92	118	140	188	284
Ammoniacal nitrogen method 2, %	0.012	0.018	0.191	0.353	0.607	0.814	1.015	1.045

Richardson and Scherubel (*J. Amer. Chem. Soc.*, 1908, 30, 1515) report ammoniacal nitrogen figures obtained by distillation of various amino acids, acid amines, and meat bases with magnesium oxide which have a bearing upon the methods given above.

Both of the preceding methods can be conducted by distillation under reduced pressure, with the advantage of lower temperatures

and consequently less hydrolysis. Distillation at ordinary pressure appears to be satisfactory, and hence it is a question to be determined by the operator whether it is worth while to resort to this further refinement of the process with its attendant complications.

(3) Pennington and Greenlee (*J. Amer. Chem. Soc.*, 1910, **32**, 561; *Z. physiol. Chem.*, **37**, 161) have applied the Folin method for the estimation of pre-formed ammonia in urine by means of an air current at ordinary temperatures to the estimation of pre-formed ammonia in flesh foods. The method cannot be commended for its simplicity, and the results obtained are comparable with the results obtained by method 1. The error most likely to affect method 1 is the production of ammonia by hydrolysis of compounds containing more firmly bound ammonia than in ammonium salts, and the method about to be discussed suffers from the difficulty of separating all the ammoniacal nitrogen in an air current. The method can be conducted as follows: 50 gm. of the finely divided meat are introduced into a litre round-bottomed flask with 300 c.c. of water and 10 gm. of recently calcined magnesium oxide. The purifying and absorption train is arranged as follows: The air is dried and freed from basic substances by drawing it through sulphuric acid in a flask provided with a Hopkins safety bulb. It then passes through the suspension of meat. The meat flask is followed by an empty 250 c.c. catchall flask, and this by the absorption flask containing  $N/10$  sulphuric acid. The last member of the train is a small empty guard flask to catch any of the standard acid mechanically sprayed over; and this flask is connected to the air pump. A volume of 8,000–10,000 cu. ft. during a period of 3 to 6 hours is necessary to remove the pre-formed ammonia. The back titration is made with the use of cochineal as indicator.

Pennington and Greenlee found that the same amount of ammonia was evolved with 0.5–2.0 gm. of sodium carbonate as with magnesium oxide, which furnishes excellent evidence that the ammoniacal nitrogen evolved by their method is well differentiated from that in the other nitrogenous compounds present in meat.

*Folin's Method.*—Folin's method (*Z. physiol. Chem.*, 1902–03, **37**, 161) for the determination of ammonia in urine by means of an air current is applicable to the estimation of ammonia in meat foods. Recent modifications are found in the literature by Folin (*J. Biol. Chem.*, 1912, **11**, 493, 523; *U. S. Dept. Agr., Bur. of Chem., Circ.* 108,

10), by Steel (*J. Biol. Chem.*, 1910, **8**, 365), and by Shulansky and Gies (*Biochem. Bull.*, 1913, **3**, 45).

**A. O. A. C. Method.**—The tentative A. O. A. C. method based on Folin's method employs a wash bottle one-fourth full of dilute sulphuric acid (1 + 9), a tube containing the sample with a rubber disc in the upper part of the tube to break the foam, a 5 c.c. bulb in the exit of this tube to prevent spray from being carried over into a tube which contains the standard acid, and finally a safety bottle. The apparatus can be seen in Fig. 12 of the *Methods (Official and Tentative Methods of the A. O. A. C., Washington, 1925, p. 238)*.

Introduce 2-4 grm. of the finely divided meat into the sample tube and add 20 c.c. of ammonia-free water. Place a measured quantity of 0.04 *N* or 0.02 *N* sulphuric or hydrochloric acid in the tube intended to receive the standard acid. Then add 1 c.c. of saturated potassium oxalate solution to the sample tube, introduce a few drops of kerosene, and finally add just sufficient saturated sodium or potassium carbonate solution to render the mixture alkaline. Place the tubes in position at once; pass air through the apparatus; and titrate the standard acid in the receiving tube at hourly intervals until ammonia ceases to be given off, using methyl red, cochineal, or Congo red indicator. If preferred, the ammonia collected may also be determined by Nesslerising.

**Amino Nitrogen.**—Amino nitrogen may be determined by Van Slyke's method (*J. Biol. Chem.*, 1911, **9**, 185; 1912, **12**, 275; 1913, **16**, 121; 1915, **23**, 407) or by Sørensen's method (*Biochem. Z.*, 1907-8, **7**, 45, 407; *Z. physiol. Chem.*, 1909, **63**, 27; 1910, **64**, 120). Brown (*J. Bact.*, 1923, **8**, 245) has modified the method of Sørensen for use in following the bacterial changes in culture media.

**A. O. A. C. Method.**—The A. O. A. C. method (*loc. cit.*, p. 248) follows Sørensen. To 20 c.c. of the filtrate from the determination of coagulable nitrogen in the water extract or 20 c.c. of a solution containing an extract of meat (in some cases a larger volume may be necessary) add 10 c.c. of a freshly prepared phenolphthalein-formol mixture. This is made by preparing 50 c.c. of commercial formol with 1 c.c. of a 0.5% solution of phenolphthalein in 50% alcohol and exactly neutralising with 0.2 *N* barium or sodium hydroxide. Titrate the mixture of extract and phenolphthalein-formol with 0.2 *N* barium hydroxide solution until a distinct red colour appears; then add a slight known excess of 0.2 *N* barium hydroxide, and titrate back

to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank determination with the same reagents, using 20 c.c. of water in place of the solution to be tested. From the quantity of 0.2 *N* barium hydroxide solution required to neutralise the mixture, corrected for the quantity used in the blank determination, calculate the quantity of amino nitrogen present (including ammonia, if this has not been removed). One c.c. of 0.2 *N* barium hydroxide solution is equivalent to 2.8 mg. of amino nitrogen.

*Sørensen Method—Brown's Modification.*—The following procedure is adapted for work with cultures or infusions of meat where the sample is held for some considerable time in order to follow the increase in the products of bacterial metabolism: With an Ostwald or serological pipette measure out 1 c.c. of the culture or infusion into each of two large test tubes. To each tube add 9 c.c. of ammonia-free distilled water or, better, sufficient water to bring the contents of both tubes to the same level. One of the tubes serves as a color screen in the comparator block. To the other tube, hereafter referred to as the sample, add 5 drops of phenol red (0.02%). Bring the reaction of the sample to pH 8.0 by the cautious addition of *N*/10 sodium hydroxide or hydrochloric acid as needed. A few drops is usually sufficient. To the sample add 4 drops more of phenol red, and then to both the sample and the colour screen tubes add 8 c.c. of formol. Twirl the sample tube just sufficiently to mix the formol with the sample and, as quickly as possible, add from a burette (graduated in hundredths of a c.c.) *N*/10 sodium hydroxide until the end-point of pH 8.0 is reached. After subtracting the titration of the formol blank from the above, the result multiplied by 10 is the formol titration expressed in terms of normal alkali. By multiplying the latter result by 14 the results may be expressed as mg. of nitrogen per 100 c.c. of medium or infusion. The results can be calculated to the original meat by knowing the relation of meat to infusion volume.

Alkaline formaldehyde solutions do not keep well. If the reaction is slightly acid, however, the solution does not change much in the course of a day. Consequently it is better to adjust the reaction to a pH of 5.0 to 7.0. The formol blank consists of 8 c.c. of the formol solution plus 10 c.c. of distilled water, which mixture is titrated with *N*/10 sodium hydroxide solution to a pH of 8.0.

**Other Chemical and Physical Methods.**—The various methods to be used in detecting incipient spoilage in meat and flesh foods have been discussed by numerous investigators. Among these are Ottolenghi (*Z. Nahr. Genussm.*, 1913, **26**, 728). Only Sørensen's method (*Biochem. Z.*, 1907-8, **7**, 45, 407) of estimating amino-acids and the microscopical examination for bacteria were found to be of practical use by him. Although Chodat's tyrosinase-*p*-cresol reaction gave reliable results, and the lowering of the freezing point and the condition of the serum also gave reliable results, they were too time-consuming and required a very elaborate manipulation. The amount of amino-acid nitrogen should not exceed 350 mg. per 100 grm. of fat- and tendon-free meat dried at 70°. Expressed on this basis, well-kept meat will usually contain 190-320 mg., and meat in an incipient state of decomposition will contain 340-700 mg. Of course, as marked decomposition approaches ammonia is formed. This ammonia interferes with the estimation and causes low results, unless first removed by distillation.

Falk (*J. Assoc. Off. Agr. Chem.*, 1924, **8**, 160) discusses meat spoilage from the standpoint of chemical composition and of the criteria desirable in chemical tests used for following changes in meat that lead to and result in spoilage. He recommends the determination of ammonia nitrogen and amino nitrogen for this purpose. The solubility of amino acids in alcohol has been used by Lüttge and v. Mertz (*Z. Nahr. Genussm.*, 1924, **48**, 451) to distinguish meat which has been rendered unfit for food through the action of saprophytic bacteria. The meat proteins are not soluble in alcohol, whilst the amino acids produced in large amounts during spoilage are soluble in alcohol of high concentration. They recommend heating 0.5-0.8 grm. of meat with 10 c.c. of 96% alcohol, filtering, and testing the filtrate qualitatively, using the ninhydrin reaction or Neuberg-Manasse reagent ( $\alpha$ -naphthyl isocyanate), or determining the amino acids by the Sørensen titration method. Any considerable amount of amino acids shows decomposition.

Almy (*J. Amer. Chem. Soc.*, 1925, **47**, 1381) has described a method of determining the hydrogen sulphide formed during the spoilage of proteinaceous food products. He gives the results of the use of the method with beef, pork, and fish and shows that the sulphide is formed progressively during spoilage of the putrefactive type.

Arbenz (*Mitt. Lebensm. Hyg.*, 1925, **16**, 84) has utilised the oxygen consumption and reduction of methylene blue for detecting and determining the approximate degree of putrefaction of meat. The procedure is much like that of Tillmans and Otto (*Z. Nahr. Genussm.*, 1924, **47**, 25). Five grm. of chopped meat are placed in a 300-400 c.c. flask which is then filled with distilled water at 22-23°, stoppered, allowed to stand for 2 hours at 22-23°, and the oxygen determined by adding 1 c.c. of 80% manganese chloride solution and 1 c.c. of 33% sodium hydroxide solution. After a few minutes some crystals of potassium iodide and 5 c.c. of concentrated hydrochloric acid are added. The free iodine is titrated with thiosulphate and the oxygen calculated. When there is no further precipitation of iodine after 2 hours, the meat is unfit for human food. For the methylene-blue test, 5 grm. of chopped meat is placed in a 60 c.c. flask, filled with distilled water at 40°, and 1 c.c. of methylene blue is added. This consists of 5 c.c. of saturated alcoholic solution to 195 c.c. of water. The flask is then placed in a 45° bath and the time for reduction noted. Less than 1 hour indicates putrefaction. Satisfactory results were obtained in testing beef, pork, veal, mutton, horse meat, mince meat, sausage, pigeon meat, and several kinds of fish.

Herzner and Mann (*Z. Untersuch. Lebensm.*, 1926, **52**, 215) made determinations of the percentage of crude protein nitrogen, pure albumin nitrogen, peptone and meat base nitrogen, ammonia and non-coagulable nitrogen compounds in meats. They found that the content of pure albumin, calculated as nitrogen in percentage of the total nitrogen, gives an indication of incipient putrefaction, the percentage decreasing with increasing putrefaction. In this connection it should be remembered that Moulton has shown (*J. Biol. Chem.*, 1920, **43**, 67) that inanition will bring about the same result. The experiments of Herzner and Mann with the flesh of the horse, ox, and pig show that the electrometric measurement of the hydrogen ion concentration, either of the meat itself or of its aqueous extract, serves as a means of detecting incipient decomposition of ripened flesh of warm blooded animals. Using a quinhydrone electrode, the value of the pH was 6.0 to 6.2 for the sound meat and above 6.2 for meat which had undergone deterioration. This method is inapplicable to meat which has been treated with chemical preservatives, as these exert an appreciable influence on the hydrogen ion concentration.

Parsons and Douglass (*J. Bacter.*, 1926, **12**, 263) have studied the salt error in the colorimetric determination of  $pH$  and have shown that with the sulphonphthalein series of indicators results in conformity with electrometric data can be obtained by subtracting 0.3 unit from the observed colorimetric  $pH$ .

In studying bacterial metabolism in meat media it is desirable to follow the changes caused by the bacteria. Methods have been devised in addition to the chemical methods by Sørensen, Folin, and others. Among these are the determination of  $pH$  and the electrical conductivity of solutions prepared from the cultures. Parsons and Sturges (*J. Bacter.*, 1926, **11**, 177; **12**, 267) have made use of the latter method and have shown that change in conductivity is closely proportional to change in ammonia and follows rather closely the change in formol titration.

Tillmans, Hirsch, and Huhn (*Z. Untersuch. Lebensm.*, 1927, **53**, 44) have studied the chemical and physical changes occurring in the incipient spoilage of meat. Extracts of fish and of cooked and uncooked meat (chiefly beef) taken from various parts of the animal were prepared by digestion of the sample with water. The physical and chemical properties of the extracts were determined daily as putrefaction proceeded. The refractive index, reduction potential, surface tension, titration in stages to various  $pH$  values, and the conductivity in the presence of acid or alkali showed no progressive alteration with time. The order of the values of the conductivity may be correlated with the amounts of electrolyte (chiefly sodium chloride) present in the sample, as indicated by the lowering of the freezing point. The colour produced with Nessler's reagent after 6 minutes and matched with potassium dichromate solution, increased progressively with the putrefaction in meat but not in fish. No substantial differences in the amounts of glycogen, purine bases, and creatinine were detected between the first and later stages of putrefaction, but the iso-nitrile reaction for primary amines was found to be a good indication of glycogen. The titration of the volatile acids and bases, separated by steam distillation of the sample with acid and alkali respectively, and the determination of their molecular weights, may be used as a guide to the progress of putrefaction in particular cases. Catalytic enzymes were found to various extents, in all the samples investigated and the beginning of putrefaction was accompanied by a great increase in their activity.

Negative results were obtained, however, in tests for diastase and proteolytic enzymes.

The question of the reaction of meat at the beginning of decay has also been studied by Schmidt (*Arch. Hyg.*, 1928, **100**, 377). He states that the onset of putrefaction in flesh may be detected more accurately from a consideration of electrometric measurements of  $pH$  than by bacteriological means, chemical methods involving the determination of ammonia, or by cryoscopic methods. The  $pH$  of horse flesh at death is 6.2. Determinations of the  $pH$  of aqueous extracts of the flesh kept at various temperatures indicate that meat with a  $pH$  of 6.1 to 6.3 may be considered un-objectable, but that meat with a  $pH$  of 6.3 or more is definitely spoiled. Herzner and Mann placed the limit at 6.2.

Katrandjieff (*Compt. rend. soc. biol.*, 1929, **99**, 112, 115) has studied the absorption of iodine by fresh and by tainted meats and has shown that the proteins in the aqueous extract increase the iodine absorption by filtrates of tainted meats. These proteins increase progressively. Also proteolytic products of the putrefactive organisms such as amino acids, ammonia and hydrogen sulphide, as well as bacteria themselves and the products of their soluble secretions, increase the iodine absorption.

According to Horowitz-Wlassowa (*Z. Untersuch. Lebensm.*, 1928, **55**, 239) the alkalinity or acidity of the aqueous meat extract, the  $pH$ , the refractive index, the biuret reaction, the total nitrogen, the nitrogen of the tannin and phosphotungstic acid precipitates, the oxidisability, and the iodine value all give no indication as to the freshness of meat. Eber's reaction frequently gives negative results in samples showing incipient decomposition. The test for ammonia with litmus paper, the meat being heated with sodium hydroxide or magnesium oxide, is of no practical value, since ammonia is split off during the test. The heating of meat extract (ground meat in ammonia-free water) for 5 minutes with the addition of magnesium oxide at 50° liberates ammonia from ammonium salts, but does not split it off from amino acids. With litmus paper this test is sensitive to 0.02% ammonia. Fresh meat does not give a positive test. The titration of amino acids by Sørensen's method gives useful indications as to the freshness of meat, but it is not as simple as the above test, since it requires the preparation of aqueous extracts. The author feels that it should be borne in mind



by the reader that small changes in the flesh may proceed for a long time after the usual day or two of chilling and still leave a wholesome edible product. Strictly fresh meat, that is, 1 or 2 days old, is in no way to be preferred to older meat properly stored. Part of Horowitz-Wlassowa's criticisms are more or less academic.

Glassmann and Rochwarger (*Z. Untersuch. Lebensm.*, 1929, 58, 585) describe a method of detecting incipient putrefaction and determining saline ammonia in flesh. According to their method fish are prepared by removing the viscera, head, and scales from 100 gm. of whole sample, which is then washed well with water and dried on filter paper; the bones are separated and the flesh is minced. In the case of meat, the sinews and fat are removed from equal portions selected from various parts of the sample, the blood is washed away, and the residue mixed and minced. The prepared sample (5 gm.) is then pounded in a mortar for 10 minutes with 50 c.c. of water, previously rendered ammonia free by the action of 0.3% of permutite for 20 minutes, the liquid is strained off and filtered, and 10 c.c. shaken for 10 minutes in a flask with 3 gm. of permutite (Folin) which has previously been shaken for 5 minutes with 5 c.c. of 2% acetic acid and washed. Fifty c.c. of water are then added, the shaking continued, and the clear liquid poured off. After repeating five times, or until the decanted liquid is clear and free from flocks, 20 c.c. of water, 5 c.c. of 10% sodium hydroxide solution, 125 c.c. of water, and 10 c.c. of Nessler solution (Treadwell) are added in succession, the mixture is diluted to 200 c.c., and the colour matched after 10 minutes with that produced by a standard solution of ammonium sulphate (1 c.c. equivalent to 0.1 mg. of ammonia) in the presence of permutite under similar conditions. Tests on samples containing known quantities of standard solution were accurate within the limits permissible for a colorimetric method. Analyses of samples made at various intervals after slaughtering indicated that 0.020 and 0.025% are the critical quantities of ammonia indicating the beginning of putrefaction in the flesh of warm-blooded animals (including poultry) and in fish, respectively.

### **Methods for the Detection of Changes in the Fat**

The fat associated with meat may also undergo characteristic changes, which, under certain conditions and when necessary precautions are observed, may give information in regard to the

condition, age, or handling of the product. The examination is made for free fatty acids and for rancidity.

**Formation of Free Fatty Acids.**—The hydrolysis of fats, with formation of fatty acids and glycerol, is brought about in fatty tissue after death by the enzyme lipase, and later, if no steps are taken to prevent putrefaction, by bacteria. In the rendering of fats, too, especially by means of steam and water under pressure, some hydrolysis occurs, so that good grades of fats and oils contain as much as 0.2 to 2.0% of free fatty acids. In the case of edible fatty tissue hydrolysis by bacteria is negligible, and therefore the fatty acids present are due to hydrolysis by the natural enzyme of the fat. This action appears to go on not only at ordinary temperatures, but even at low temperatures in tissues which are well supplied with the enzyme. The lipase of hog pancreas is moderately active at a temperature of  $-12^{\circ}$  or even lower, but experiments made with beef fat and leaf fat from swine indicate that the natural lipase is inactive at such low temperatures or is not present in sufficient quantity to produce a measurable effect. The fats were taken within 24 hours after death and held at  $-9^{\circ}$  to  $-12^{\circ}$ , and analyses made from time to time up to 2 years. The amount of free acid varied from 0.3 to 0.6%, depending upon the sampling and rendering, at the beginning and throughout the experiment. In some varieties of summer sausage and also in old dried hams ("Italian" style ham, "Virginia" ham, "Westphalian" ham, etc.) the free fatty acids in the fat may rise to 9-10%.

**Estimation of Free Fatty Acids.**—If possible, the fat tissue should be separated from the lean of the sample, hashed, and rendered at a low temperature in a porcelain dish in the steam bath or over a low flame. The melted rendered fat is then filtered. This is the best method for freeing the fat from organic impurities. Ten to twenty grm. of this fat are then titrated in hot neutral alcohol against standard alkali, with phenolphthalein as indicator. The result is calculated either to acid value (mg. of KOH per grm. of fat) which is preferable, or by conversion, to percentage of oleic acid (mol. wt. 282).

If the fat is distributed throughout the lean and cannot be separated by hand, it may be extracted by means of ether or petroleum spirit after drying the sample, but this introduces the possibility of two errors: first the acids formed by oxidation during the drying (supposing this to be done in the air); second the extraction of organic

acids from the lean by the solvent. These latter, if they are water soluble, can be removed by washing in a separator or on a wet filter with hot water.

Pennington and Hepburn (*J. Amer. Chem. Soc.*, 1910, **32**, 569) have proposed making the free acid estimation on the fatty tissue directly without rendering, but this is not to be recommended except in cases where the quantity of the sample is too small to admit of rendering. They hash the fatty tissue, place the weighed sample ( $\pm 10$  grm.) in an Erlenmeyer flask, boil with alcohol neutral to phenolphthalein, and titrate against standard alkali using the same indicator. The end-colour is fugitive and its persistence for  $\frac{1}{4}$  minute is taken as the end-point. The calculation is made as previously described.

**Examination for Rancidity.**—Rancidity is a condition of fats resulting from oxidation. It is characterised by the production of volatile substances of disagreeable odour. This action goes on in the air at ordinary temperatures, but it is enormously accelerated by sunlight and to a considerable extent by heat. Inversely, it is retarded by low temperatures and storage in the dark. The process is purely a chemical one in which bacteria are not concerned, although under some conditions it may be aided indirectly by bacterial action. This may occur when fats or fatty tissue are emulsified with water containing bacterial nutritive substances.

Moisture (the humidity of the air is sufficient) appears to be essential to rancidity, and it is likely that the oxidation affects principally fatty acids following hydrolysis of the fat. Glycerol is not subject to rancidity, although when fats turn rancid the glycerol in *statu nascendi* appears to be further split. In ordinary examples only a small part of the fat is affected or altered by the process. The products which are formed are largely lower fatty acids and aldehydes, free alcohols, and esters, and as these are volatile, a fat exposed to air and sunlight continually loses weight, unless the effects of rancidity are more than counterbalanced by the drying process in which oxygen is absorbed from the air.

The characteristic odour, to an experienced chemist, affords the best evidence of rancidity. This odour is distinctive, although not identical in all cases, and unmistakable, but difficult to describe.

A chemical test for rancidity has been proposed by von Raumer (*Analyst*, 1897, **22**, 265), but the test is of limited usefulness and it

is doubtful whether it affords positive evidence of rancidity, since over-heated fats respond to it. Schiff's reagent, prepared as follows, is used: 40 c.c. of a 5% solution of fuchsin (magenta) are mixed with 250 c.c. of distilled water and the dye is bleached by adding 10 c.c. of sodium hydrogen sulphite solution (sp. gr. 1.375) and 10 c.c. of sulphuric acid (sp. gr. 1.84). After standing the mixture becomes colourless. Five c.c. of the rendered, melted fat are mixed with 10 c.c. of the reagent, heated to 90–100°, and shaken. Rancid fats give a deep red to violet colour to the reagent, which does not fade, whilst fresh fats give none, or at most a faint pink which fades in 30 minutes in the cold. When rancid fat is distilled with steam, volatile aldehydes, as well as the lower fatty acids, come over and can be tested according to the usual methods.

According to Kreis (*Chem. Ztg.*, 1904, **28**, 956) if the melted rancid fat is shaken with hydrochloric acid (sp. gr. 1.19) and 1% phloroglucinol solution in ether, a red colour (due to aldehydes and ketones) is produced. The ether used must be free from peroxide.

Powick (*J. Agr. Research*, 1923, **26**, 323) has discussed at length the compounds developed in rancid fats and has made observations on the mechanism of their formation. He also gives an excellent review of the literature. He states, in brief, that the odour of heptylic aldehyde is sufficiently suggestive of the rancid odour to establish the reasonableness of Scala's contention that it is the component of rancid fats responsible for the rancid odour. Nonylic aldehyde may be partly responsible. The colour of the Kreis test is spectroscopically the same as that found in the Kreis test for mixtures of acrolein and hydrogen peroxide, which gives epihydrin aldehyde. This latter is not free in fats showing the Kreis test, but is probably present as an acetal of epihydrin aldehyde. The test is not always a reliable one for rancidity, as other compounds give the colour.

### Bacteriological Methods

While bacteriological methods lie outside the scope of this work, some discussion of the general principles and the value of the methods is desirable.

Fellers, Hunter, and Korff (*Amer. J. Public Health*, 1929, **19**, 389), in a report to the American Public Health Association, have

pointed out that it is extremely difficult to tell with accuracy when food is spoiled or decomposed and that there are all degrees of spoilage. Apart from organoleptic tests, such as appearance, odour, and taste, few really satisfactory criteria or tests are known, especially those which would be valuable in incipient or border-line cases. Chemical methods of estimating certain decomposition products are known. These have been discussed above. The following discussion is based on the report of Fellers, Hunter and Korff.

Weinzirl (*Amer. J. Public Health*, 1924, **14**, 946) discusses the relation of bacterial count to the putrefaction of meat and concludes that aerobic and facultative bacteria assist in spoilage of meat, but that the putrefactive odour is mainly due to anaerobes. The aerobic count is, therefore, only indirectly an indication of putrefaction. It does, however, indicate certain types of spoilage and the sanitary conditions under which meat is kept. A test for anaerobes in meat, comparable with the *B. coli* test in water analysis, appears highly desirable. Weinzirl describes the spoilage process as a complex one, including: (1) the souring of meat due to fermentation of carbohydrates by aerobic, facultative, and anaerobic bacteria; (2) a digestion of the proteins by aerobes and facultative anaerobes without an abundant evolution of sulphides; (3) the production of indol, skatol, and other vile-smelling compounds by aerobic, facultative, and anaerobic bacteria; and (4) putrefaction with the production of sulphides by anaerobes.

Certain bacteria are able to produce compounds such as ammonia, hydrogen sulphide, indol, amino acids, trimethylamine, and free fatty acids. Some species may form all of them, yet others are incapable of producing any deep-seated changes in foods, and to the latter group so many of the food-poisoning organisms belong. A number of authors (Weinzirl and Newton, *Amer. J. Public Health*, 1914, **4**, 408 and 413; Cary, *ibid.*, 1916, **6**, 124; Le Fevre, *Am. Food J.*, 1917, **12**, 140; Hoffstadt, *Amer. J. Hygiene*, 1924, **4**, 33 and 43; Brewer, *J. Bact.*, 1925, **10**, 543) have shown that meat is often grossly contaminated with bacteria, but that there appears to be no clear-cut correlation between the quality of the meat and the bacterial count. The Weinzirl anaerobic spores test (*Amer. J. Public Health*, 1921, **11**, 149) is useful, and Hoffstadt states that the presence of proteolytic anaerobes is a definite way by which the keeping qualities of meat can be predicted. Apart from the anaerobic spore test

and total counts of bacteria, little of value has been obtained by bacteriological means.

It should be borne in mind that the mere presence of these bacteria is of little value in predicting the keeping qualities or condition of the product. In the laboratories of the Institute of American Meat Packers, Lewis and Jensen have shown that the relative numbers of bacteria per grm. are an excellent indication of the keeping qualities of Frankfurter style sausage. Storage conditions provide a factor of importance. A low bacterial count and proper storage conditions, especially temperature, will insure a good product, whilst higher counts may lead to difficulties, such as discolorations in Frankfurters, before any other sign of damage is shown.

The report of Fellers, Hunter and Korff, after discussing the various biochemical methods of detecting spoilage, points out that the work of Weaver (*Michigan Agr. Expt. Station, Tech. Bull.* 79, 1927), in which the production of hydrogen sulphide after incubation of the meat in nutrient broth was used as an indication of incipient spoilage, is the most outstanding piece of work of the two years 1927 and 1928. In concluding, the report states that if simple, rapid laboratory or field tests for the detection of incipient decomposition in meats, fish, and shell fish can be devised, they will be of untold value not only to the public health laboratory worker or field inspector, but also to the handlers of these products and to the general public as well.

**Some Specific Organisms Responsible for Spoilage.**—Those responsible for the handling of meats in the packing plant have long been interested in determining what organism or organisms are responsible for the various types of spoilage encountered.

In the case of the so-called ham souring or spoilage of hams which may occur in the cushion of the ham or along the bone, especially at the aitch bone joint, the *Clostridia* are involved. McBryde (*U. S. Dept. Agr., Bur. An. Ind., Bull.* 132, 1911) found an organism which he classed as *Bacillus putrefaciens* (*Clostridium putrefaciens*). Boyer (*Amer. Food J.*, 1923, 18, 197; *J. Agr. Research*, 1926, 33, 761) isolated *Bacillus putrefaciens*, *B. histolyticus*, *B. sporogenes*, *B. tertius*, and an organism resembling *B. oedematiens* in some respects. Moran (Moulton, *Meat through the Microscope*, p. 155) isolated *Clostridium sporogenes*, and Tucker has isolated *Cl. putrificum*. Sturges and Reddish (*J. Bact.*, 1926, 11, 37) and Sturges and Par-

sons (*J. Bact.*, 1926, **11**, 189) have reported the isolation of an organism, which they have named *Cl. flabelliferum*, from a table on which hams were handled and from certain salts and meats cured with these salts.

Roderick and Norton (Moulton, *Meat through the Microscope*, p. 170) have shown that a coccus form of bacterium is responsible for slimes on sausages such as Frankfurters.

Moulds are a frequent menace to meats. Lewis and Yesair (Moulton, *Meat through the Microscope*, p. 188) have made an extended study of moulds in the packing plant and list some twelve types found there, including *Penicilium expansum*, three types of *Aspergillus*, *Mucor racemosus*, *Monascus purpureus*, *Alternaria tenuis*, and *Oidium lactis*. Wright (*J. Soc. Chem. Ind.*, 1923, **42**, 488 T) has shown that "black spot" of frozen meats is due to a mixture of moulds. Brooks and Kidd (*Dept. Sci. Ind. Research, Food Investigation, Special Report No. 6*, 1921) had claimed the causative agent to be *Cladosporium herbarum*, while Wright found that both *Mucor mucedo* and *Penicillium glaucum* were also involved. Brooks (*J. Soc. Chem. Ind.*, 1924, **43**, 306 T) repeated some of his experiments and found only *Cladosporium herbarum* to be involved in "black spot." Brooks and Kidd found that other organisms and moulds may accompany the *Cladosporium*. These include: white moulds, species of *Sporotrichum* and *Oospora*; bluish-green moulds, *Penicillium* species; pink yeasts; and whiskers, species of *Mucor* and *Thamnidium*. Wright (*New Zealand J. Sci. Tech.*, 1921, **4**, 74) reports that a red spot on frozen meat is due to *Bacillus prodigiosus*, a brown spot to a yeast, and a black spot to *Oidium carnis*.

The "sour beef," due to acid fermentation during storage while warm under conditions which do not allow proper cooling, is caused by *Bacillus megatherium*, according to Bunyea (*J. Agr. Research*, 1921, **21**, 689).

### **Poisonous Meat, Meat Poisoning, Ptomaine Poisoning**

In the discussion of unsound meat in these pages the presence and detection of animal parasites are purposely not considered, since, with the possible exception of inspection for trichinae, the subject would be out of place in a work of this character. For complete information on the subject consult Ostertag: *Handbuch der Fleisch-beschau*, or the English translation: Ostertag-Wilcox, *Handbook of*

*Meat Inspection*; also Edelmänn, Mohler, and Eichhorn: *Meat Hygiene*.

There are a number of different cases coming under this heading which may be classified as follows:

(1) Flesh rendered poisonous by contamination with mineral or organic poisons.

(2) Flesh made poisonous by the food of the animal.

(3) Flesh made poisonous by metabolic products of the cells of the living animal.

(4) Flesh made poisonous by the presence of pathogenic bacteria in the living animal.

(5) Flesh made poisonous by bacteria after death of the animal (so-called ptomaine poisoning).

**(1) Flesh Rendered Poisonous by Contamination with Mineral or Organic Poisons.**—The cases of this sort, which sometimes occur, come properly within the scope of toxicology. Reference should be made to a standard work on this subject, as detailed methods of analysis cannot be given in this place. Methods for estimating metals and their compounds which may be absorbed by contact, especially from metallic containers, are given under "Canned Meats." Tin is the metal most commonly to be looked for; other metals possibly present are zinc, copper, lead, arsenic, and antimony.

**(2) Flesh Made Poisonous by the Food of the Animal.**—Not only do food animals consume food which makes meat derived from them unpalatable, but sometimes also actually poisonous. For vertebrates such food may be colchicum, equisetum, lupines, buckwheat (intermingled with the fodder) or sprouting potatoes, ricinus, mustard cake, etc. (Ostertag-Wilcox, *Handbook of Meat Inspection*, 379. See also Letheby, *On Foods*, 221). Shell-fish and poisonous tropical fish also appear to derive their poisonous nature in certain cases from their food (Letheby. Also Guenther, *The Study of Fishes*, 189). (For a list of poisonous fish see Andrews, *Handbook of Public Health*, 1898, 51.) Flesh foods may also be made poisonous occasionally by the animal's accidental eating of definite mineral or organic poison (lead, phosphorus, arsenic, etc.) or by improper medication (tartar emetic, mercury, strychnine, carbolic acid, etc.) of the animal (Walley, *Meat Inspection*, 57. Ostertag-Wilcox, *Handbook of Meat Inspection*, 379. Mitchell, *Flesh Foods*, 216). The animal may or may not be killed or made sick by the food or poison.



(3) **Flesh Made Poisonous by Metabolic Products of the Cells of the Living Animal.**—In the catabolic processes of the animal cell, many protein degradation products are formed, before the end-products urea, carbon dioxide, water, etc., are reached. Many of these substances are basic and nitrogenous and related chemically to the ptomaines (see below under *Flesh Made Poisonous by Bacteria after Death*); some, indeed, as choline, neurine, betaine, tri-methylamine, neuridine, cadaverine, etc., are identical with the putrefaction bases (Mitchell, *Flesh Foods*, 218; Vaughan and Novy, *Cellular Toxins*). These metabolic products of the living cell are known collectively as leucomaines. Most of them are harmless or nearly so, but on the other hand, some are decidedly poisonous (neurine, choline). Normally they are soon eliminated and do not accumulate in the tissues in excess. The predisposing cause for the presence of undue amounts of leucomaines in the flesh of higher animals is over-fatigue, for example in over-hunted game, hard-driven cattle, and animals which have struggled violently after having been caught in a trap. An occasional illness caused by eating the flesh of hard-hunted game may be due to leucomaines (Gautier, *Les Toxines*, 1896, 438, 455, 456).

(4) **Flesh Made Poisonous by the Presence of Pathogenic Bacteria in the Living Animal.**—Two cases may be distinguished under this head: first, those due to specific bacteria which existed and occasioned disease in the animal before slaughter. Such an organism is the *B. enteritidis* of Gaertner. These cases have, in times past, occurred not infrequently in Germany (consult Ostertag) following cases of "emergency" slaughter of diseased and dying animals. It seems almost incredible that such meat should be eaten at all, even by a half-starved population, and doubly incredible that it should be eaten without adequate preliminary cooking.

There are also many cases on record wherein the various bacteria of pyaemia and septicaemia, from animals afflicted with these diseases at the time of slaughter, caused sickness among the consumers of the meat (Mitchell, *Flesh Foods*, London, Griffin, 1900, 220).

Second, are those cases of sickness caused by pathogenic bacteria which are present in or on the flesh food in question, but which produced no disease in the living animal from which it was derived. The best-known examples of this class are botulism caused by

*Cl. botulinum* and those cases due to the contamination of shellfish in waters polluted by sewage (*U. S. Dept. Agri. Bur. Chem. Bull.* 136, 1911. This bulletin contains an excellent bibliography). Typhoid fever and Asiatic cholera are the diseases most to be feared from this source. The bacteria *B. coli* and *B. enteritidis sporogenes* have been found abundantly present in such food, although they are not normally present in the common shellfish, or in sea water uncontaminated by sewage.

Botulism or sausage poisoning, also called *allantiasis* and *ichthyosism*, caused by *Cl. botulinum* of van Ermengem, requires special mention. This disease is the most typical and the best known of those caused by pathogenic bacteria which are also capable of living on meat products as *saprophytes*. It has been of most frequent occurrence in those parts of Germany and Saxony where it is customary to eat meats such as ham and sausage in raw condition. The period of incubation usually varies from 18 to 48 hours. Fatal cases result in death in from 4 to 8 days. In the past as many as 25 to 30% of the cases have proved fatal, but the disease is now rarely observed. *Cl. botulinum* is an anaerobe and develops especially in meat products whose preparation—pickling, smoking, ageing, drying—favours *anaerobiosis*. The bacteria may develop in rather closely circumscribed areas, and hence some parts of the infected meat may be quite innocuous; others decidedly injurious. The symptoms of the disease may be summed up as central nervous disturbances, secretory disturbances, and motor paralysis. Fever is absent. Van Ermengem's conclusion that the disease is caused by toxins preformed in the meat (not formed in the body) does not harmonise with the fact that an incubation period intervenes before the onset of the characteristic symptoms, and requires further investigation. As stated, the disease is now rare and thorough cooking appears to obviate the danger (*Cf. Report in Analyst* 1923, 48, 118).

**(5) Flesh Made Poisonous by the Action of Bacteria after Death of the Animal (So-called Ptomaine Poisoning).**—The expression "ptomaine poisoning" is commonly used by the physician and the public to designate almost any sort of sickness caused by the eating of spoiled food. The word ptomaine was brought into the literature in the years 1874 to 1877 by Selmi before the doctrine of bacterial toxins had been developed, to designate poisonous, basic, sometimes crystalline, bacterial products, to which the injurious action of

bacteria was ascribed. In the bacterial degradation of the proteins a long series of simpler basic substances is produced, some harmless and some harmful. Among these relatively simple basic substances are those which Selmi named the ptomaines or animal alkaloids, and of these some are very poisonous and cases of meat poisoning from eating putrid or decomposing meat have undoubtedly been due to them. Some of them are not decomposed by boiling water, but retain their toxicity even after boiling for some time; thus cooking does not render them harmless, and this property distinguishes them from the bacterial toxins, none of which withstands a boiling temperature. The toxins proper (*ectotoxins*) appear to be elaborated within the bacterial cell and are excreted into the surrounding medium. Thus they are both soluble and diffusible. Contrasted with these are the so-called *endotoxins*, which are elaborated within the cell and remain therein. Thus, three classes of poisons, all of bacterial origin, must be considered in connection with meat poisoning.

The knowledge that a water extract of decomposed animal matter had poisonous properties is very old, and older still is the knowledge that spoiled food might prove toxic. Yet the action of spoiled foods is by no means uniform. A food which decomposes spontaneously—that is by uncontrolled bacterial action—may prove harmful at one time and not at another, or harmful to one person and not to another. Meat which is even very slightly tainted will cause severe gastro-intestinal symptoms when eaten by some individuals, whereas meat in which bacterial decomposition is much more advanced may be eaten by preference by others and cause no ill effects whatever. Such so-called “gamey” meat is a regular article of diet by certain epicures.

More striking still are certain nitrogenous foods which are allowed to undergo a more or less controlled putrefaction, or other fermentation, for the purpose of developing strong flavours. These foods are very generally eaten and appear to be entirely harmless. Important among them are the strong cheeses, such as Roquefort and Limburger. Fish is eaten in a state of incipient decomposition by certain islanders. A preparation of eggs which have undergone a controlled putrefaction is consumed by certain Chinese, while in the Dardanelles, fish-roe cheese is prepared by allowing the roe to undergo a fermentation and pressing and drying the product in the air.

It is plain that the facts in regard to the harmless consumption of decomposed nitrogenous food greatly complicate the subject of ptomaine poisoning. The conditions which give rise to it are necessarily somewhat obscure. It appears certain that not every putrefactive fermentation of flesh foods gives rise to poisonous ptomaines, at least in the earlier stages of decomposition, while the product is still fit for food, and in some cases if they are formed, they appear to be fugitive, disappearing a little later. Thus it has been observed that a flesh food poisonous at one period of its history may become harmless subsequently. Nor is there positive evidence that ptomaine poisoning is caused by a specific bacterium or by a limited number of specific bacteria.

There can be no doubt that in recent years, since the properties of toxins have become established, attention has been directed away from Selmi's ptomaines as the active agencies in cases of food poisoning. It is likely that attention will again have to be directed to them in order to account for many of the obscure minor cases of food poisoning, in which the gastro-intestinal symptoms follow quickly after the ingestion of the food—too quickly to admit of an incubation period which is characteristic of the best known and most typical kind of bacterial poisoning, botulism, caused by *Cl. botulinum*.

The following table of the ptomaines and basic degradation products of proteins is due to Mitchell (*Flesh Foods*) and follows Gautier's scheme of classification (See Vol. VIII, pp. 193 and 207, and Abderhalden, *Biochemische Untersuchungsmethoden*, 1909, 2, 1002-1042. The recent edition is called *Handbuch der biochemischen Arbeitsmethoden*).

*Monamines of the Aliphatic Series.*

Trimethylamine,  $(\text{CH}_3)_3\text{N}$ , herring pickle.

Di-ethylamine,  $(\text{C}_2\text{H}_5)_2\text{NH}$ , putrid meat extract.

Tri-ethylamine,  $(\text{C}_2\text{H}_5)_3\text{N}$ , decomposed cod-fish.

Propylamine,  $\text{C}_3\text{H}_7\text{NH}_2$ , decomposing cod-liver.

Butylamine,  $\text{C}_4\text{H}_9\text{NH}_2$ , decomposing cod-liver.

Amylamine,  $\text{C}_5\text{H}_{11}\text{NH}_2$ , cod-liver oil.

*Diamines of the Aliphatic Series.*

Putrescine, or Tetramethylene-diamine,  $\text{C}_4\text{H}_{12}\text{N}_2$ . Putrid horse flesh.

Cadaverine, or pentamethylene-diamine,  $C_5H_{14}N_2$ . Putrid fish and blood.

Neuridine,  $C_6H_{14}N_2$ , putrid meat, albumin, gelatin.

Saprine,  $C_5H_{14}N_2$ , decomposed flesh.

*Guanidines.*

Methylguanidine,  $C_2H_7N_3$ , putrid horse flesh and beef.

*Aromatic Ptomaines, Free from Oxygen.*

$\beta$ -Phenylethylamine, putrid gelatin.

Parvoline,  $C_9H_{13}N$ , putrid horse flesh after several months.

Corindine,  $C_{10}H_{15}N$ . Putrid cuttle-fish.

Di-hydrocollidine,  $C_8H_{13}N$ , putrid fish and horse flesh.

*Oxygenated Ptomaines.*

Neurine,  $C_5H_{13}ON$ , putrid meat on fifth or sixth day.

Choline,  $C_5H_{15}O_2N$ , accompanies neurine.

Muscarine,  $C_5H_{15}O_3N$ , putrid fish.

Betaine,  $C_5H_{11}O_2N$ , in mussels (leucomaine).

Homopiperidinic acid,  $C_6H_{11}O_2N$ , decomposition of meat fibrin.

Mytilotoxine,  $C_6H_{15}O_2N$ , in poisonous mussels.

Mydatoxine,  $C_6H_{13}O_2N$ , putrid horse flesh after nine to fifteen months.

Gadinene,  $C_7H_{18}O_2N$ . (Putrid fish, especially cod.)

Methylgadinene,  $C_8H_{20}O_2N$ .

Unnamed base of Brieger,  $C_7H_{17}O_2N$ , accompanies mydatoxine.

*Aromatic Oxygenated Bases.*

Tyrosamines,  $C_7H_9NO$ ,  $C_8H_{11}ON$ ,  $C_9H_{13}ON$ , decomposing cod-liver.

Mydine,  $C_8H_{13}ON$ , decomposing human flesh.

*p*-Hydroxyphenylethylamine, cheese, cod-liver, etc.

According to Geiger (*California and West. Med.*, Nov., 1929, as quoted in *J. Amer. Med. Assoc.*, 1930, **94**, 1554), the scientific worker generally recognises two types of food poisoning. One type is due to contamination of the food with paratyphoid-enteritidis group (*Salmonella-suispestifer* group) or other bacterial organisms, either through the agency of a human or animal carrier or from meat of an animal suffering from a specific infection with these germs.

Subsequent incubation of the contaminated food through improper and insufficient cooking, refrigeration, or storage, allows the bacteria to secrete, in their growth, a poisonous product, or, perhaps in the process of heating, certain products become soluble and evidently poisonous. The consumption of such food is followed within several hours by symptoms of nausea, abdominal pain, vomiting, prostration, diarrhea, and perhaps fever. Complete recovery within forty-eight hours is the rule. The other type of food poisoning is known as botulism. It is due to the contamination of the food with a specific bacterium known as *Bacillus botulinus* (*Cl. botulinum*). Bacteriological examination and the testing for toxins by means of laboratory animals (such as guinea pigs or mice) are the only methods for the examination of foods to determine the presence of these organisms.

The Journal of the American Medical Association (*J. Amer. Med. Assoc.*, 1930, 95, 36) has summed up the modern point of view in an editorial as follows:

"The expression 'ptomaine poisoning' has been for the most part abandoned in medical discussions and diagnosis during the last few years. Ptomaine was the term introduced by Selmi about 1850 and applied to a group of basic substances, having alkaloidal properties, which were obtained from decomposing proteins and were highly toxic to animals, when injected. Presently, however, the rapidly developing knowledge of bacteria and their possible rôle in the genesis of disease directed attention to the microbiotic aspects of the subject.

"As Damon has pointed out, since the studies were made that directed attention to the possibility that bacteria might be the etiologic agents in causing illness in man in cases that had previously been regarded as ptomaine poisoning, the bacteriologic investigations of many workers have widened our field of knowledge and shown that a variety of bacterial species are capable of growing in foodstuffs under widely different conditions, and that they must be held accountable for many, if not nearly all, cases of food poisoning formerly attributed to ptomaines. In fact, Damon notes further, at the present time improved methods of detection are more and more frequently indicating that infection of food is responsible for illness in those who consume it. It thus seems probable to Damon that practically all food poisoning, in which illness is not induced by a

hypersensitiveness of the individual or by a metallic poison, or in which an alkaloid of vegetable origin is not involved, is due to infection.

"Thus the bacterial theory of food poisoning has gradually supplanted the older and somewhat less specific ptomaine or chemical theory. Perhaps it would be more in accord with actual practice to say that investigators of cases of food poisoning, as the present nomenclature expresses it, seek the presence of pathogenic bacteria or their products in the food under suspicion. There are increasing indications that toxic material as well as noxious bacteria need to be taken into consideration. Indeed, this may in some ways represent a return to the older chemical theory, with the exception that the *materies morbi* is directly attributed to the metabolism of well-defined and recognised micro-organisms. This is exemplified in the recent report of Jordan of the University of Chicago. Incriminated food secured during an outbreak of food poisoning contained a yellow staphylococcus in considerable abundance. This apparently produced a toxic substance in broth, as shown by the effect of the sterile filtrate, which, on being swallowed by a human volunteer, gave rise to nausea, diarrhea, and prostration of the same character as that observed in the original outbreak. Furthermore, Jordan has now demonstrated that various strains of staphylococci of diverse origin and different cultural characters are capable of generating in broth a substance which, when taken by mouth, produces gastro-intestinal disturbance. This substance is destroyed by boiling and is either destroyed or greatly weakened by being heated at from 60° to 65° C. for thirty minutes. It seems not unlikely to Jordan that bacteria of other groups may produce similar substances that are irritating to the human alimentary tract. From the standpoint of recognising the immediately harmful factor in many instances of food poisoning, the development of toxic derivatives deserves new emphasis. It is fortunate that such toxic substances usually lose their potency when suitably heated."

### Examination of Supposed Unsound Meat Products

Passing judgment upon a sample of meat which is supposed to have been the cause of sickness is one of the most difficult tasks which confronts the chemist. No general rules applicable to all

cases can be formulated, but the following statements can be made which will assist the investigator in arriving at a conclusion.

(1) If the meat was consumed in fresh condition—that is, not salted or pickled, and if it was well cooked, and if it appears from the examination to be sound and untainted (absence of bacteria in interior, low ammoniacal nitrogen, etc.) the conclusion would be that the meat was harmless, unless it could be included under the exceptional cases 1, 2, or 3 given above.

(2) If the meat was consumed in fresh condition but uncooked, suspicion should attach to it in any case. Thorough inspection before and after slaughter reduces the danger to a minimum; but fresh meat should never be eaten raw.

(3) If the meat was salted or pickled and cooked just before consumption, in nearly all cases it could not be considered harmful. On the other hand, if cooked at the time of manufacture and then stored for a longer or shorter period, further investigation should be made. The results of ammoniacal nitrogen determinations and Eber's sulphide test are not so conclusive in the case of salted and pickled meats as with fresh meats.

(4) If the meat was salted or pickled and consumed in the raw state, it may always be looked upon with suspicion until further examination proves it to be innocuous.

The analyst who examines meat products which are suspected of having caused illness, should be familiar with the appearance, odour, and flavour of the various kinds of fresh and cured meats and meat products in sound condition. No rules or descriptions can be laid down which will be in any sense the equivalent of individual experience with the various products.

Sound meat derived from mammals properly slaughtered and properly handled and stored should be reasonably firm to the touch (not flabby) and should scarcely moisten the finger. It often has a marbled appearance, especially in the case of young fat animals, due to the distribution of fatty tissues between the muscle bundles. It should have the characteristic odour of the species but no disagreeable odour. The presence of excessive moisture (above 74%) is often recorded as an indication of spoiled meat, but it is doubtful whether this idea is well founded, and if it were, before the increase in moisture could be noticed or determined, other criteria would point certainly to decomposition.



As to colour, there are considerable differences to be observed, depending upon the species and age of the animal, the location of the cut and the method of slaughter. An animal killed by bleeding yields lighter-coloured meat, than by other methods. Typical beef has a good red colour and is neither pale nor dark purple. Veal and pork are much lighter in colour, partly due to the method of slaughter. Some birds possess dark muscular tissue only; others, as the domestic fowl, both dark and light. Fish muscle is generally white, but in some species a pink or reddish colour prevails. All of these points must, however, be learned by the analyst from careful inspection.

After the physical examination which should take note of colour, odour, texture, and the other general characteristics and condition of the sample (fresh, salted, smoked) the chemical examination proper is begun. All or a part of the following determination and tests may be made.

- (1) Examination for metals. (See Canned Meats.)
- (2) Determination of ammoniacal nitrogen (page 323).
- (3) Application of Eber's sulphide test (page 323).
- (4) Free acid and rancidity of fat (pages 334, 336).
- (5) Examination for ptomaines.
- (6) Bacteriological examination.

These tests have all been considered previously, with the exception of the last two. If decomposition has advanced to the point where ptomaines are formed, evidence of decomposition will be disclosed by the first three tests. If a specific examination for ptomaines is called for, the investigator is referred to the methods in Vaughan and Novy's Cellular Toxins, as the methods, which are seldom used at present, demand more discussion than can be given here.

The bacteriological examination requires the expert services of a bacteriologist. The methods presume thorough knowledge of that science and can only be briefly outlined here.

Feeding experiments are important and should be conducted with a number of species of animals, such as guinea pigs, rabbits, mice, rats, dogs, and cats. If any of the animals become sick, the symptoms should be noticed, and, if death ensues, the body should be examined for pathogenic bacteria. Negative results do not necessarily indicate absence of pathogenic bacteria. According to Dr.

E. C. Novy the further examination should proceed as follows (*Bull.* 107 (revised), *U. S. Dept. Agri. Bur. Chem.*, 1908): "Another set of animals should be injected with a cold extract of the meat made with sterile water. If the animals die, they are to be examined for pathogenic bacteria. A third set of animals should receive similar injections, though larger portions, of this aqueous extract which has been previously filtered through sterile porcelain. If the animals die from such injections, the same as with unfiltered solutions, it is evident that a soluble bacterial chemical poison is present.

"The identification of the toxin produced by the germ is wholly out of the question. The most that can be done satisfactorily is to obtain, as above, a germ-free solution of the poison (see Abderhalden, *Biochemische Untersuchungsmethoden*, Vol. 2, p. 1002).

"A bacteriological examination proper should be made of the original poisonous meat and of all the animals that died either from eating the meat or from the injections of the aqueous extracts. The organism present in the animal, if any, must be isolable directly from the meat. If it happens, as it sometimes has, that the dead animals contain no germs, it is proof that they were killed by a toxin elaborated by a germ in the meat previous to the injection. Cultures from the meat will then reveal the germ, and the effects of its pure cultures should correspond to those observed with the poisonous meat.

"To prepare the cultures from the original food, the latter should be cut out with a sterile knife and the material should be taken from the inside, thus avoiding all chances of contamination.

"Several sets of beef-tea tubes and agar plates should be made. One set should be set aside in a Novy anaerobic jar at room temperature; a second similar set should be placed at a temperature of 37°. A third set should be grown in the presence of air at room temperature, and a like set at a temperature of 37°."

The following is a good technique to follow in taking samples of meat for bacteriological examination. The surface of the meat is first seared with a Bunsen burner. If the sample is to be cut across, a complete circle is seared around the sample in this way. The section is then made by means of knives (and saws if necessary, as in the case of pork hams) sterilised by boiling in 3% sodium carbonate or by heating in the naked flame. At the place where a sample is to be taken the cut surface is again seared by a branding iron in the

form of a circular disc 1 or 2 in. in diameter, and the sample cut out by means of sterile scalpels. These pieces can be dropped at once into sterile culture media, or can be further cut up with suitable precautions. Preferably, all these operations are to be carried out under a glass case entirely closed except for a moderate sized opening on the side facing the operator.

In passing judgment on meat food products for soundness or unsoundness and excluding cases 1, 2, and 3 given above, and thus including unsoundness due to bacterial influences only, consideration should be given to the following points: Ordinary bacterial decomposition or putrefaction of meat may or may not render it harmful, but such meat should always be looked upon with suspicion. Incipient decomposition (gaminess, *haut goût*), may be without effect or may cause gastro-intestinal disturbances resulting in slight purging or rather violent diarrhoea. These results may occur in from 8 to 24 hours and are usually, but not always, of short duration. On the other hand, advanced decomposition (putrefaction) even after the product has been cooked, will invariably cause violent symptoms and possibly death in the average subject. Besides ordinary saprophytes and their products there may be present representatives of two different classes of pathogenic bacteria, of which *B. enteritidis* and *B. botulinus* are typical, and which may occasion serious specific diseases and death. Thus meat which is slightly decomposed can never be said to be positively harmful; yet it is always to be looked upon with suspicion. This appears to be the unavoidable conclusion, although the harmlessness of such meat in the majority of cases is a matter of everyday record.

## PRESERVATION OF MEAT

### Methods

There are four general methods used for the preservation of meat and other foods. They are: desiccation, heat sterilisation, low temperatures, and the use of chemical substances which inhibit bacterial growth. This latter method, as applied to meat, is called curing and may involve smoking. The use of strict antiseptics is largely prohibited, especially in the United States of America, both by law and custom. However, meats sometimes have to be examined for preservatives or antiseptics.

Exclusion of atmospheric air is often given as a method of preservation, but, at best, it is only a partial means and very imperfect. It acts by creating conditions unfavourable for the development of aerobic micro-organisms, but does not prevent the development of facultative and anaerobic organisms, and as these together outnumber the exclusively aerobic organisms, putrefaction is not prevented and not greatly retarded. In the absence of air (or, more accurately, oxygen) rancidity, which is a chemical (not bacterial) oxidation of fats, does not occur, and therefore exclusion of air is a most valuable adjunct to the methods enumerated above, and in combination with these produces the best-known methods for the preservation of flesh foods.

### Desiccation

This is one of the oldest and most effective methods for the preservation of all kinds of foods, including meat, and has been used from a time long antedating written history. It is the method most commonly used by savage and semi-civilised tribes at the present day, and naturally so on account of its simplicity and effectiveness. Ordinary fresh meat as prepared and stored in large establishments possesses a dry surface, and this is an important point for its preservation. The surfaces of cured meats such as hams, bacon, smoked beef, and sausage are also maintained in dried condition, and the drying is quite as important for their preservation as the smoking, if not more so. Certain cured meats, such as so-called "Italian" hams and summer sausage (*Cervelat*, *Salami*, etc.), are purposely hung to dry for a long period, after which they remain in good condition indefinitely. *Jerked meat*, *biltong*, *charque*, *carne secca*, *carne Tassajo* are names applied to dried meat in different parts of the world. The method of preparation usually consists in cutting the meat in thin strips and drying in the sun or over a slow fire, with or without previously coating the product with salt, sugar, or flour. Meat powder and dried blood (Sweden) are also articles of commerce.

Pemmican is a preparation of meat made by the American Indians, chiefly from buffalo or deer flesh or fish. The meat was cut into strips, dried, and reduced to a powder in a mortar. About an equal quantity of fat was then incorporated with it. The modern product,

as used chiefly by explorers in cold regions, usually has a fruit, such as currants, added.

Dried fish are prepared by splitting them down the middle and drying in the air, by civilised and uncivilised peoples. Smoked fish, which are more or less desiccated, are also common articles of commerce.

The chief characteristic of desiccated meats is, of course, the absence of much moisture. Fresh lean muscular tissue contains about 75% water, and as water is the first requisite for the growth and reproduction of bacteria, it is natural that a considerable reduction in this quantity makes the product a poorer medium for bacterial growth and thus insures its preservation to a greater or lesser extent. On the other hand, dried meat products are apt to become rancid, on account of the fat they contain, when exposed to the air, and this is especially true of comminuted products where a large surface is exposed.

### Heat Sterilisation

Heat sterilisation has been a matter of knowledge, more or less imperfect, ever since the art of cooking foods was practised, but the contamination of such sterile foods by exposure to sources of bacterial infection as the air was not fully understood until recently. However, in a practical way, the process of preservation by heat sterilisation accompanied by sealing in hermetically closed containers was disclosed by Appert in 1809. His original process provides for heating the product in earthenware vessels and protecting it from subsequent infection by hermetic sealing. It has been recognised that no air should be left above the product for the best results, and this has been brought about variously by completely filling the containers, as with jelly, fats, or oils, by leaving the space filled with steam, which later condenses leaving a vacuum, and by filling the space with an inert gas such as nitrogen or carbon dioxide. Appert's process in one or another modification has been used for preserving practically all kinds of food and is used at present in large canning establishments for the production of immense quantities of tinned vegetables, fruits and meats, including fish.

Heat sterilisation of meats is based on the following facts: The vegetative forms of micro-organisms in moist condition are all killed below the temperature of boiling water, and their spores are

killed at temperatures not higher than  $120^{\circ}\text{C}$ . corresponding to a gauge steam pressure of 14.7 pounds. In canning factories, heat sterilisation is known as "processing" and is carried out in autoclaves called "retorts." The steam pressures used generally lie between 4 and 10 pounds, as shown on the gauge.

The principal kinds of sterilised canned meats are boiled beef, corned beef, tongue, canned chicken and poultry, and various deviled meats and pastes. Among sterilised canned fish, salmon, sardines, and other small fat fish are the principal varieties.

The preservation of meat (and other kinds of food) by confining it in hermetically sealed vessels is now practised on an enormous scale. In the case of meat, the material, freed from bone, is packed in the tins, and an addition of jelly or gravy, salted and flavoured, is often made. The tins are then closed, with the exception of a small orifice, and processed. The current of steam issuing from the tin through the aperture left for the purpose carries the air with it, and the high temperature effectually destroys any lower organisms. When the air is judged to be thoroughly expelled, the orifice is closed by solder. When the operation is carefully performed, the contents of the tin will keep in a good condition for an indefinite period.

There are other methods of evacuating the can. One is to seal or crimp on the lid in a vacuum sealing machine under 22 to 25 inches of vacuum. A second method is to process the material in a retort and seal soon after removal. A modification of this method is to run the filled can without a top through a steam exhauster which may bring the contents of the can up to  $115$  to  $135^{\circ}\text{F}$ ., and then to seal on the lid immediately in a closing machine. A third method is to seal, place in boiling water, remove after the contents of the can are hot, and then puncture the lid and reseal by soldering. The method of using the steam exhauster and closing while hot is a very common one. The final sterilisation process is carried out in retorts for sufficient lengths of time and at high enough pressures of steam to destroy heat resistant spores such as those of *Cl. botulinum*, the heat resistance of which is usually taken as a standard. However, many meat-canning processes greatly exceed that temperature. Commercial experience seems to indicate the desirability of the heavier processes.

A new line of canned meats has recently come onto the market. Examples are: cured whole and half hams (boned) and spiced ham or cured luncheon loaf or other cured product. These meats are fairly fat and cannot be sterilised without rendering the product unmerchantable. Reliance is, therefore, placed on adequate curing of the product, a mild processing in retort or water-bath, and subsequent storage under refrigeration. Some of these newer products seem to be but little affected by high temperature storage. However, the flavour and quality are deteriorated by warm temperatures. This fact, and the apparent need of refrigeration to insure a safe product for consumption, make storage of these products under refrigeration imperative unless and until other means can be devised to meet the situation. The education of the distributor and consumer to the necessity of refrigeration is a necessary step in successful merchandising of these products.

For a description of meat-canning processes see *Bulletin 13, Part 10, U. S. Dept. Agr. Bureau of Chemistry*; C. N. McBryde, *Annual Report U. S. Bureau of Animal Industry, 1907*, p. 270; Richardson, *Encyclopedia of American Agriculture, 3*, 261; *U. S. Dept. of Agriculture, Bureau of Chemistry, Bulletin 151*, 1912.

Tinned meat, preserved in the foregoing manner, is sometimes over-cooked, though this fault has been less evident of late years. Occasionally, through imperfect sealing of the tin, the contents undergo change, and when there is any evidence of this they should on no account be eaten. Incomplete sterilisation will result in gradual bacterial fermentation of the meat, with production of gas, and sometimes with formation of poisonous products. Hence any can which is bulged by internal pressure, or from which gas issues on opening, should be rejected.

H. A. Baker (8th Int. Cong. App. Chem., New York, 1912, Section VIIIc, Bromatology) states that "springers" is a trade term given to cans with bulging ends which contain perfectly sound and sterile food products. The gases in the head space of "springers" are never more than three: carbon dioxide, nitrogen, and hydrogen. Very often no hydrogen is found. Oxygen is practically never found. The carbon dioxide is formed during the time of processing, or when the product is not worked through quickly from the beginning of preparation to final sterilisation, or when it is allowed to stand in the container before sterilisation. Nitrogen is simply a

residue from unremoved air. Hydrogen when formed is the product of attack of organic acids on the metallic container. "Springers" are usually produced in warm weather, but can be avoided by suitable precautions. They are more apt to occur in the case of vegetable products than in the case of meat products.

Occasionally, severe and even fatal ptomaine poisoning has occurred by the use of decomposed canned foods, but such cases bear such a small proportion to the enormous number in which the meat is found good and wholesome, that, with care in the directions above named, danger from this cause is very remote.

#### **Analysis of Canned Meats.—**

The chemical examination of canned meats requires in certain cases, besides the usual determinations described under "General Methods of Analysis of Meats," an examination of the gases contained in the can and an examination for metals—especially poisonous metals—derived principally from contact with the metal package.

The gases can be collected by means of the apparatus of Doremus (*Amer. Chem. J.*, 1897, **19**, 733).

A bevelled, hollow steel needle is attached to the upper arm of an adjustable clamp. The point and lower part of the shaft are covered by a rubber stopper, which serves as a soft pad. The lower arm is moved along the body of the clamp until the can to be pierced is held between the rubber stopper and the head of the screw. The upper part of the needle is connected by means of a capillary tube, filled either with water or mercury, with a receiver also filled with either of these liquids. The receiver may be a stop-cock eudiometer or a nitrometer.

The apparatus adjusted, a turn or two of the screw clamps the can tightly between the rubber-pad at the top and the screw-head

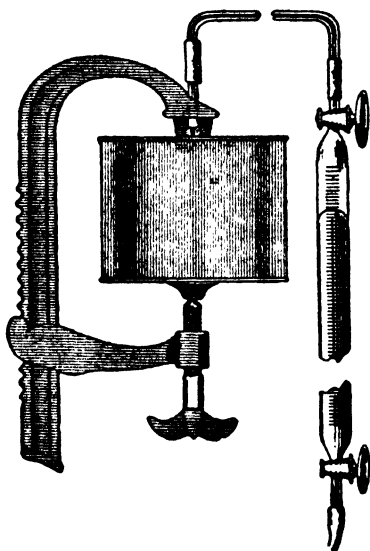


FIG. 21.—Apparatus for collecting the gases of canned foods.



below. The rubber yields to the pressure, making a tight joint around the needle. When the latter pierces the tin, the contained gases of the can escape gently into the eudiometer.

H. A. Baker (*8th Int. Cong. App. Chem.*, New York, 1912, Section VIIIC, Bromatology) employs an apparatus with perforating needles both at the bottom and top of the can. Through the lower needle he introduces a current of water (under about 15 lb. air pressure) which sweeps the gas in the can under examination through the upper perforating needle and into a gas burette. With this apparatus Baker has investigated the disappearance of oxygen in canned food containers in an endeavour to explain the fact that oxygen is practically never found in the "head space" of cans in which food is packed, although slight traces have been found in cans containing foods of less than two months' age. The analyses usually show carbon dioxide 8 to 15%, with the balance nitrogen. Hydrogen is also found to be present in some instances, particularly with acid fruits. Baker concludes that oxygen disappears in tinned food containers in at least the three following ways:

- (1) By combining with the metals tin and iron, forming oxides.
- (2) By oxidising tin or iron salts.
- (3) By combination with nascent hydrogen, when organic acids act on the metallic container.

For the analysis of the separated gases reference should be made to *Hempel's Gas Analysis* (translated by L. M. Dennis), or to other standard works on the subject. The gases, methane, hydrogen, nitrogen, carbon dioxide, carbon monoxide, and oxygen are among those to be expected. Nitrous oxide, ammonia, and hydrogen sulphide are also among the possibilities, although they would naturally be present in very small quantity.

Savage (*Dept. Sci. Ind. Research, Food Investigation Board, Special Report No. 3*, London, 1920) gives an excellent critique of the methods used for the inspection of canned meats and their reliability for this purpose. The methods have to do with visual inspection, palpation, percussion, and shaking the unopened can, as well as inspection and bacteriological examination of the contents.

**Composition of Canned Meats.**—On account of the cooking it has received the meat proper in the can will contain less moisture than uncooked meat, but the can, as a whole, may contain more

water than would correspond to the water content of uncooked meat (see **Cooking of Meat**, p. 313).

Warden and Bose have published (*Chem. News*, 1890, 61, 304) some unusually complete analyses of typical samples of canned beef and mutton. They found the moisture to range from 49 to 57%; the fat from 10 to 22; the proteins (*i. e.*,  $N \times 6.25$ ) from 24.5 to 29; the ash from 0.62 to 4.36; the chlorine from 0.11 to 2.65; the phosphoric acid from 0.31 to 0.40; the hot-water extract from 5.35 to 10.14%, with a content of nitrogen ranging from 0.88 to 1.10%.

In making these analyses, the entire contents of a can were thoroughly pulped in a large mortar, great care being taken to scrape the interior of the can free from fat and jelly. The plan of taking a slice of the contents and regarding that as a fair sample Warden regards as fallacious.

For the determination of *moisture*, a weight of from 5 to 6 gm. of the sample was teased with forceps in a flat platinum dish, and dried first at 100° and subsequently at 120°. The samples were then moistened with absolute alcohol and redried. The whole time of heating occupied from 8 to 9 hours. In another large platinum dish from 30 to 40 gm. weight of pulp was similarly heated, reduced to fine powder, and again heated. This dried pulp was preserved in a closely stoppered bottle and employed for the determination of fat, nitrogen, and aqueous extract. The ash and ash-constituents were conveniently determined on the undried pulp.

In estimating the *ash*, the portion of the pulp used for ascertaining the moisture was charred at a temperature below redness, crushed with a glass rod, exhausted with boiling water, and again ignited. The residue was again treated with boiling water, and the insoluble ash ignited and weighed. The aqueous extract was evaporated to dryness, the residue heated nearly to redness, and weighed to find the soluble ash. The total ash was regarded as the sum of the soluble and insoluble ashes determined as just described, and it was found that the figures thus obtained agreed well with determinations of the total ash by direct ignition, while avoiding the difficulty experienced in the latter case in effecting complete combustion of the carbon without losing a portion of the alkali-metal salts by volatilisation.

The soluble ash was used for the estimation of *potassium* and *sodium*, by dissolving it in water and adding in succession barium chloride, ferric chloride, and ammonia to the warm solution, the last reagent being employed in quantity sufficient to render the liquid just alkaline. The precipitate (consisting of barium sulphate, ferric phosphate and ferric hydroxide) was filtered off, the filtrate treated with ammonium carbonate and ammonium oxalate, and warmed for some time on the water-bath. The precipitate (consisting of barium carbonate and calcium oxalate) was removed by filtration, the filtrate evaporated to dryness in platinum, and the residue gently ignited. The residue was redissolved in water, the solution filtered from a little barium carbonate, the filtered liquid treated with a drop of hydrochloric acid, and evaporated with platinic chloride to effect a separation of the potassium and sodium. According to Richardson the use of perchloric acid in such cases is greatly preferable (Davis, *J. Agric. Sci.*, 1912).

For the estimation of the *chlorine* and *phosphoric acid*, Warden and Bose mixed 20 grm. of the freshly pulped meat with about 2 grm. of pure sodium carbonate, dissolved in sufficient water to cover the pulp. The resulting magma was evaporated to dryness, carbonised, extracted successively with water and with nitric acid, the residue again ignited and dissolved in nitric acid, and the chlorine and phosphates estimated in the mixed solution by the usual methods.

The *total nitrogen* was estimated in the dried pulp by Kjeldahl's process, and multiplied by the factor 6.25 to find the proteins.

The *extractive matter* was estimated by boiling 1 grm. of the dry pulp with distilled water in a 100 c.c. flask, and when cold diluting to 100 c.c. The liquid was passed through a dry filter, and an aliquot portion of the very faintly opalescent filtrate evaporated to dryness in a platinum dish, and the residue weighed. The greater part of the filtrate was used for the estimation of extractive nitrogen by Kjeldahl's method.

The *fat* was estimated by treating about 0.5 grm. of the dried pulp in a small accurately-stoppered weighing bottle, and adding a measured volume of light petroleum spirit from a burette. The mixture was allowed to stand for 2 days, with occasional agitation, when a portion of the perfectly clear supernatant liquid was withdrawn by a small burette, and a carefully measured volume discharged into a small beaker. The petroleum spirit was then distilled

off, and the residual fat dried at 100° and weighed. This method, which is due to Dragendorff, was found by Warden and Bose to give results which agreed closely with those obtained by exhausting the substance with a solvent of fat in the usual way.

Warden and Bose have compared their analyses of canned meats examined by the foregoing process with the figures obtained by König by the analysis of fresh beef and mutton (p. 218). They find that, while the percentage of moisture in the canned meat is usually lower than in fresh meat, the fat in canned meat as a rule exceeds the proportion in fresh. Taking the nitrogenous matters as representing the nutritive value of the meat, and ascertaining their amount by multiplying the total nitrogen by 6.25, they obtain the following amounts of albuminous matters in the anhydrous and fat-free samples of meat examined:

	Albuminous matters in anhydrous, fat-free meat %
Average of canned beef samples . . . . .	87.06
Average of canned mutton samples . . . . .	87.19
Average of all fresh cow- and ox-flesh . . . . .	93.94
Average of all fresh mutton . . . . .	93.81
Average of all canned meat samples . . . . .	87.12
Average of all fresh meat samples . . . . .	93.87

Analyses by König of 7 specimens of canned meat showed them to have the following average composition: proteins, etc., 28.97; fat, 12.63; ash, 3.71; and water, 54.69%. These figures correspond to 10.33% of nitrogen and 27.27% of fat in the anhydrous samples, and to 88.63% of albuminous matters in the anhydrous and fat-free samples.

From the foregoing figures Warden and Bose conclude that canned meat has a sensibly lower nutritive value than fresh meat. The apparent difference is, however, increased by the salt which has evidently been added to some of the canned meat samples.

*Preservatives* in canned foods can be sought for and estimated by the methods given on pp. 426, 440 *et seq.*

The following analyses by J. König show the percentage composition of commercial potted foods. The foie-gras paste was obtained from Strassburg, and the remaining samples from Crosse & Blackwell, London.

	Water	Nitrogenous matters	Nitrogen-free extract	Fat	Ash	Common salt
Foie-gras paste.....	46.04	14.59	2.67	33.59	3.11	0.22
Potted beef.....	32.81	17.17	3.36	44.63	2.03	.....
Potted ham.....	25.57	16.88	.....	50.88	6.78	5.72
Potted tongue.....	41.52	18.46	0.46	32.85	6.71	5.98
Potted salmon.....	37.64	18.48	0.70	36.51	6.67	5.65
Potted lobster.....	51.33	14.87	4.04	24.86	4.90	0.38
Anchovy paste.....	36.81	12.33	5.18	1.59	44.09	40.10

The accompanying table is compiled from Atwater and Bryant's figures (*U. S. Dept. Agric. Exp. Sta. Bull. 28*, (Rev.), 1906).

#### COMPOSITION OF CANNED MEATS—ATWATER AND BRYANT

Food materials	Number of analyses	Re-fuse %	Water %	Protein		Fat %	Total carbohydrates %	Ash %	Fuel value per pound, cal.
				N × 6.25 %	By difference %				
Beef, Canned									
Boiled beef, as purchased.....	1	.....	51.8	25.5	24.4	22.5	.....	1.3	1,425
Cheek, ox, as purchased.....	1	.....	66.1	22.2	22.3	8.4	.....	3.2	765
Chili-con-carne, as purchased.....	1	.....	75.4	13.3	13.3	4.6	4.0	2.7	515
Collaps, minced, as purchased.....	1	.....	72.3	17.8	17.9	6.8	1.1	1.9	640
Corned beef:									
Minimum.....	15	.....	43.2	20.7	19.6	11.7	.....	2.0	1,000
Maximum.....	15	.....	58.3	35.1	34.2	31.1	.....	7.3	1,695
Average.....	15	.....	51.8	26.3	25.5	18.7	.....	4.0	1,280
Dried beef, as purchased:									
Minimum.....	2	.....	44.2	38.0	37.1	6.1	.....	9.8	955
Maximum.....	2	.....	45.3	40.4	40.1	4.8	.....	12.6	965
Average.....	2	.....	44.8	39.2	38.6	5.4	.....	11.2	960
Kidneys, stewed, as purchased:									
Minimum.....	2	.....	70.9	14.6	.....	4.9	.....	2.1	580
Maximum.....	2	.....	72.9	22.1	.....	5.4	4.3	2.8	620
Average.....	2	.....	71.9	18.4	.....	5.1	2.1	2.5	600
Luncheon beef, as purchased.....	1	.....	52.9	27.6	26.4	15.9	.....	4.8	1,185
Palates, ox, as purchased:									
Minimum.....	2	.....	69.6	16.4	15.9	9.4	.....	0.4	750
Maximum.....	2	.....	73.1	19.3	19.0	10.6	.....	2.0	755
Average.....	2	.....	71.4	17.8	17.4	10.0	.....	1.2	755
Roast beef, as purchased:									
Minimum.....	4	.....	55.8	20.3	19.3	9.0	.....	1.2	935
Maximum.....	4	.....	62.8	29.8	30.0	23.6	.....	1.4	1,375
Average.....	4	.....	58.9	25.9	25.0	14.8	.....	1.3	1,105
Rump steak, as purchased.....	1	.....	56.3	24.3	23.5	18.7	.....	1.5	1,240
Sweetbreads, as purchased.....	1	.....	69.0	20.2	19.5	9.5	.....	2.0	775
Tails, ox:									
Edible portion.....	1	.....	67.9	26.3	24.6	6.3	.....	1.2	755
As purchased.....	1	29.7	47.7	18.5	17.3	4.5	.....	0.8	535

COMPOSITION OF CANNED MEATS—ATWATER AND BRYANT.—  
(Continued)

Food materials	Number of analyses	Re-fuse %	Water %	Protein		Fat %	Total carbohydrates %	Ash %	Fuel value per pound, cals.
				N $\times$ 6.25 %	By difference %				
Tongue, ground, as purchased:									
Minimum.....	6	.....	42.5	20.1	20.2	21.6	.....	2.9	1,305
Maximum.....	6	.....	54.9	23.6	22.8	32.6	.....	5.1	1,750
Average.....	6	.....	49.9	21.4	21.0	25.1	.....	4.0	1,455
Tongue, whole, as purchased:									
Minimum.....	5	.....	42.4	10.8	18.6	15.7	.....	3.0	865
Maximum.....	5	.....	57.4	23.4	23.0	32.7	.....	6.3	1,725
Average.....	5	.....	51.3	19.5	21.5	23.2	.....	4.0	1,340
Tripe, as purchased:									
Minimum.....	2	.....	68.9	16.5	16.2	2.6	.....	0.4	425
Maximum.....	2	.....	80.2	17.0	16.6	14.5	.....	0.6	920
Average.....	2	.....	74.6	16.8	16.4	8.5	.....	0.5	670
Lamb, Canned									
Tongue, spiced and cooked:									
Edible portion.....	1	.....	67.4	13.9	14.3	17.8	.....	0.5	1,010
As purchased.....	1	2.6	65.7	13.5	13.9	17.3	.....	0.5	980
Mutton, Canned									
Corned, as purchased:									
Minimum.....	1	.....	45.8	28.8	27.2	22.8	.....	4.2	1,500
Tongue, as purchased:	1	.....	47.6	24.4	23.6	24.0	.....	4.8	1,465
Pork, Canned									
Brawn, boars' brains, as purchased:									
Minimum.....	2	.....	44.3	20.1	18.2	12.0	.....	4.3	1,110
Maximum.....	2	.....	53.7	30.3	28.5	33.2	.....	4.9	1,775
Average.....	2	.....	49.0	25.2	23.4	23.0	.....	4.6	1,440
Boars' heads, as purchased:									
Minimum.....	2	.....	50.5	19.8	17.8	19.3	.....	2.8	1,180
Maximum.....	2	.....	60.1	21.6	20.7	25.0	.....	3.8	1,455
Average.....	2	.....	55.3	20.7	19.2	22.2	.....	3.3	1,320
Ham, deviled, as purchased:									
Minimum.....	6	.....	38.4	16.5	16.9	29.5	.....	2.3	1,610
Maximum.....	6	.....	49.4	21.4	20.5	38.9	.....	4.4	1,975
Average.....	6	.....	44.1	19.0	18.5	34.1	.....	3.3	1,790
Poultry and Game, Canned									
Chicken, sandwich, as purchased.....	1	.....	46.9	20.8	20.5	30.0	.....	2.6	1,655
Turkey, sandwich, as purchased.....	1	.....	47.4	20.7	20.7	29.2	.....	2.7	1,615
Plover, roast, as purchased.....	1	.....	57.7	22.4	.....	10.2	7.6	2.1	985
Quail, as purchased ..	1	.....	66.9	21.8	.....	8.0	1.7	1.6	775

More recent work has been done on canned meats by Wiley and Bigelow (*Bull. 13, Part 10, Bur. Chem. U. S. Dept. Agric., 1902*).

Wright and Bevis (*New Zealand J. Sci. Techn., 1926, 8, 163*) have reported the composition of canned tongue, canned mutton, canned roast beef, and canned corned beef. Their analyses include the water extracts, and the methods used were essentially the A. O. A. C. methods.

**PERCENTAGE COMPOSITION OF CANNED TONGUE—WRIGHT AND BEVIS**

	Sheep tongues				Ox tongues	
	Fresh	Canned			Fresh	Canned
Water.....	69.46	65.56	62.49	65.21	68.30	56.09
Ether extract.....	14.71	13.59	16.90	18.68	11.46	14.37
Ash.....	1.21	3.04	3.30	1.54	1.18	5.07
Salt.....		2.24	2.38	0.87		3.74
Total phosphorus.....	0.19	0.16	0.16		0.19	0.17
Total nitrogen.....	2.32	2.65	2.52	2.15	2.90	3.76
Insoluble nitrogen.....	1.66	1.87	1.68	1.83	2.06	2.85
Amino nitrogen.....	0.22	0.06	0.18	0.17	0.34	0.22
Ammonia nitrogen.....		0.02	0.02	0.03		0.03
Acidity, as lactic acid.....	0.17	0.18	0.18	0.20	0.17	0.19
Saponific. value of fat.....	199.0	204.7	196.4	195.0		189.0
Melting point of fat.....	27.2°	20.4°	24.0°	34.1°		36.6°

**PERCENTAGE COMPOSITION OF CANNED MUTTON AND BEEF—WRIGHT AND BEVIS**

	Mutton				Beef				
	Fresh	Canned		Fresh	Canned				
		Boiled	Corned		Roast	Corned			
Water.....	68.24	59.71	50.55	69.16	56.98	55.20	55.85	59.83	
Ether extract.....	12.08	20.19	18.99	11.53	15.87	15.95	13.48	7.28	
Ash.....	1.06	1.89	2.93	1.28	1.36	3.52	4.59	3.33	
Salt.....		0.98	2.18		0.60	2.82	3.29	2.27	
Total phosphorus.....	0.15	0.22	0.20	0.17		0.19		0.18	
Total nitrogen.....	2.92	4.36	4.33	2.85	3.93	4.41	4.11	4.81	
Insoluble nitrogen.....	2.25	3.19	3.17	2.11	2.87	3.41	3.43	3.50	
Amino nitrogen.....	0.26	0.23	0.20	0.32	0.38	0.18	0.28	0.21	
Ammonia nitrogen.....	0.04	0.04	0.02	0.02	0.03	0.02	0.02	0.04	
Acidity, as lactic acid.....	0.58	0.25	0.36	0.62	0.27	0.27	0.20	0.45	
Saponific. value of fat.....	196.0	194.1	203.6	193.0	196.8	212.2	199.2	193.2	
Melting point of fat.....	43.0°	42.6°	42.4°	42.2°	40.2°	43.8°	40.6°	38.0°	

Wright and Forsyth (*New Zealand J. Sci. Techn.*, 1926, 8, 305) report the composition of a product known as hearts and skirts, which is used as a canned meat.

**PERCENTAGE COMPOSITION OF HEARTS AND SKIRTS (WRIGHT AND FORSYTH)**

Water.....	58.53	Total nitrogen.....	3.69
Fat.....	13.94	Amino nitrogen.....	0.18
Ash.....	3.71	Ammonia nitrogen.....	0.03
Salt.....	0.88	Acidity, as lactic acid.....	0.18
Phosphorus.....	0.20	Saponification value of fat..	191.10
Protein.....	23.06	Melting point of fat.....	41.0° C.

**Metals in Canned Foods.**—Tin may be dissolved from the containing can rather readily in the case of acid fruits, and sometimes to such an extent as to communicate a distinct metallic taste to the food. Lacquers have been developed to protect the can and the product from such action, as well as to prevent certain discolorations which sometimes occur. Canned meat, soup, and fish are less liable than fruits and vegetables to contamination from this cause, but the evidence on the subject is very conflicting. Thus, J. Attfield (*Pharm. J.*, iii, 14, 719) failed to find more than very minute traces of tin compounds in various canned foods, but states that he not infrequently detected minute particles of metallic tin by carefully washing the external surfaces of a mass of meat just removed from a can. Out of 50 cans of preserved animal food, G. W. Wigner (*Analyst*, 1880, 219) found only one to contain tin in appreciable quantities.

On the other hand, A. E. Menke (*Chem. News*, 1878, 38, 971) detected and determined tin in canned pineapple, apple, and lobster. In 1880, G. J. Wishart (*Chem. News*, 42, 47) found tin in canned pineapples, apples, and greengages, in quantities ranging from 0.21 grain to 0.36 grain of  $\text{SnO}_2$  per 2-pound can, together with much larger proportions of iron. The taste was distinctly metallic, and the fruits were uneatable. In 1883 A. Wynter Blyth found tin in every one of 19 samples of canned fruit (apricots, pineapples, tomatoes), the proportions ranging from 15 to 11 grains per pound.

In 1889 Sedgwick showed that poisonous effects were produced by pears which had been cooked in a tinned saucepan. Beckurts, in the same year, called attention to the formation of tin sulphide by the action of albuminous matters on tin, and Nehring recorded the presence of tin in preserved asparagus in quantities ranging from 0.19 to 0.31%. Bettink, in 1890, found from 19 to 72 mg. of tin per kgrm. of tinned lettuce and meat which had occasioned the illness of a number of soldiers. Kayser found 0.19% of tin in preserved eels which had proved injurious to several persons who had partaken of them. Allen found 0.21% of tin in tomato-sardines suspected to have occasioned colic and diarrhœa.

The amount of tin dissolved necessarily depends on the length of time the article of food had been in contact with the metal, but van Hamel Roos, in an extensive research on the subject, found all tinned foods, whether of animal or vegetable nature, to contain more or less tin (abst. *Analyst*, 1891, 195).



O. Hehner (*Analyst*, 1880, 219) found canned animal foods of almost every variety to contain more or less tin. The weight of tin found in one of the soups was 0.035 grm. in a 1-pound can; in a can of preserved milk, 0.008 grm.; and in a 1-pound can of preserved oysters 0.045 grm., in addition to a considerable quantity of copper. Copper has been found to occur naturally in oysters. The tin was found throughout the mass of the soups and pasty foods, but in the case of the hard meats existed chiefly on the surface. In many cases the cans were much discoloured and blackened on the inner surface, but in others the surface of the metal was perfectly bright, although there was an abundance of tin in solution.

Tin is not usually regarded as a very active poison, but much evidently depends on the condition, as to solubility or otherwise, in which the metal is present, and whether it be in the stannous or the stannic condition. Hehner found that freshly precipitated and moist stannous hydroxide, when given to a guinea-pig, acted as a powerful irritant poison, whereas freshly precipitated and moist stannic hydroxide was comparatively inert.

The action of tin on the animal organism was systematically investigated by T. P. White (*Pharm. J.*, iii, 17, 166), who concluded "that tin, though possessing decided toxic properties when introduced into the blood, is entirely devoid of danger when taken internally in any form that could arise from being in contact with fruit or vegetables."

In a case recorded by Luff (*Brit. Med. J.*, April 12, 1890), preserved cherries contaminated with tin appeared to act as an irritant and cardiac poison.

For the detection of the tin in the above analyses, Hehner incinerated about 30 grm. of the material in a platinum basin, and heated the ash with strong hydrochloric acid. The greater part of the acid was then boiled off, about 30 to 40 c.c. of water added, and the liquid filtered. The alternate treatment of the residue with acid and water was repeated until no more tin could be extracted. The clear (and, as a rule, colourless) solutions thus obtained were then treated with hydrogen sulphide, and any yellow precipitate of stannic sulphide further treated, if necessary, in the usual manner.

In the foregoing process it is assumed that boiling hydrochloric acid can be relied on to dissolve tin from the ash of food, but according to Allen its complete solution is always difficult and

sometimes impossible to effect. It is highly probable that the negative results obtained by some chemists when examining canned foods for tin (*e. g.*, J. Attfield, *Pharm. J.*, iii, 14, 719) have been due to the use of inefficient methods of analysis.

Bigelow and Bacon (*Circular 79, Bur. Chem. U. S. Dept. Agric.*) call attention to the fact that while it has been customary to attribute the presence of tin salts in canned foods to the action of acids, several varieties of canned foods, such as fish, beets, lima beans, asparagus, pumpkin, and shrimp, although of low acid content, have a marked solvent action upon the tin lining of the container in which they are preserved. Their results, as shown in the following tables, indicate that some agent other than acid is present and exerts a pronounced solvent action on the tin. In the case of shrimps they believe this substance to be monomethylamine, and they suggest that this or a similar compound (amine or amino-acid) may be at work in other canned goods. Their conclusions are not astonishing in view of the well-known solvent action of alkalies and bases on tin.

ACIDITY AND TIN CONTENT OF CANNED GOODS ABOUT 6 MONTHS AFTER PACKING—BIGELOW AND BACON

Substance	Acidity as acetic acid, %	Milligrams of tin per kilogram	Milligrams of tin per 100 mgrm. of acid
Salt fish.....	0.012	90	75.0
Salt fish.....	.012	112	93.3
Beets.....	.036	262	72.8
Corn.....	.012	33	27.5
Corn.....	.012	46	38.3
Pumpkin.....	.012	193	26.8
Lima beans.....	.017	16	21.2
String beans.....	.108	80	7.4
String beans.....	.108	98	9.1
Pumpkin.....	.156	117	7.5
Pumpkin.....	.156	93	6.0
Corn.....	.019	12	6.3
Peas.....	.126	69	5.5
Peas.....	.126	57	4.5
Peas.....	.025	13	5.2
Pears.....	.180	86	4.8
Pears.....	.180	79	4.4
Raspberries.....	.450	194	3.9
Tomatoes.....	.390	290	7.4
Tomatoes.....	.390	145	3.7
Tomatoes.....	.234	84	3.6
Cranberries.....	.534	180	3.3
Cherries.....	.966	146	1.5
Peaches.....	.486	90	1.9
Grapes.....	.510	61	1.2
Plums.....	.582	63	1.1

## ACIDITY AND TIN CONTENT OF OLD CANNED GOODS—BIGELOW AND BACON

Substances	Average of sample in years	Acidity as % acetic acid	Milligrams tin per kilogram.	Milligrams tin per 100 mgrm. acid
Yellow beets.....	Over 3	0.05	725	145
String beans.....	Over 10	.04	551	138
Corn.....	10	.11	503	51
Succotash.....	Over 3	.10	444	44
Mock turtle soup.....	Over 5	.10	306	30
Asparagus.....	2 to 3	.13	331	26
Tomatoes.....	16	.43	944	20
Peaches.....	Over 3	.41	786	19
Apples.....	Over 5	.22	364	17
Red kidney beans.....	Over 10	.23	313	14
Blackberry jam.....	8	.31	383	12
Roast beef.....	Over 10	.33	426	11
Beans (baked).....	18	.34	388	11
Apricots.....	Over 3	.49	487	10
Lima beans.....	8	.19	170	9
Greengages.....	Over 3	.69	519	8
Apple butter.....	Over 5	1.05	690	7

Emery (*Annual Report U. S. Bur. Animal Ind.*, 1909, page 265) has studied the effects of edible fats and oils upon metallic containers and concludes that under certain conditions, such as high acidity, presence of moisture, air, and heat, metals such as iron, tin, lead, and copper are attacked to a considerable extent. The conditions within a can are nearly the reverse of those described, and hence any great amount of corrosion would not be looked for.

**Estimation of Metals in Canned Foods.**—The following methods are suitable for the estimation of tin and certain other metals in organic products.

Allen devised the following process. With obvious modifications, the method can readily be applied quantitatively. The substance to be examined for heavy metals is placed in a porcelain capsule, and concentrated pure sulphuric acid dropped on it and incorporated with the aid of a glass rod. The acid should be in sufficient quantity to moisten the substance, but an excess should be avoided. The dish is then heated on a water-bath for a short time, after which the temperature is gradually raised to complete the decomposition of the chlorides. About 1 c.c. of strong nitric acid should now be added, and the heating continued till red fumes are evolved. Ignited magnesia in the proportion of 0.5 gm. for each c.c. of sulphuric and nitric acid previously used is now gradually added and incorporated with the material. The dish containing the mixture is then ignited at a *dull red* heat, preferably in a gas-muffle. After cooling, the ash is moistened with nitric acid and then gently re-ignited, this treat-

ment being repeated till the carbon is wholly consumed. The residue is then treated with 8 to 10 drops of strong sulphuric acid, heated till fumes are evolved, cooled, boiled with water, diluted, *without filtration*, to about 100 c.c. and hydrogen sulphide passed through the liquid to saturation. The solution is then filtered, and examined according to the following scheme of analysis:

<b>Aqueous Solution</b> may contain zinc, iron, earthy phosphates, etc. Add bromine-water to destroy sulphuretted hydrogen and ensure the existence of any iron in the ferric state, boil, then add excess of ammonia, boil again, and filter.	<b>Precipitate and Residue</b> may contain PbS, SnO <sub>2</sub> , SnS <sub>2</sub> , CuS, CaSO <sub>4</sub> , etc. Fuse in porcelain for 10 minutes with 2 grm. of mixed potassium and sodium carbonates and 1 grm. of sulphur. When cool, boil with water and filter.		
<b>Precipitate</b> may contain iron, phosphates, etc.	<b>Filtrate</b> , if blue, contains nickel. Divide into two portions.		<b>Residue</b> . Boil with strong hydrochloric acid as long as hydrogen sulphide is evolved, add a few drops of bromine-water to complete the oxidation of the copper sulphide and filter if necessary. To the filtrate add excess of ammonia, when a blue coloration will be indicative of copper. Acidify the liquid with acetic acid and divide into two portions.
	I. Heat to boiling and add potassium ferrocyanide. White precipitate or turbidity indicates <b>Zinc</b> .	II. If zinc be found in I., for its determination acidify the ammoniacal solution strongly with acetic acid, filter if necessary, and precipitate the zinc from the filtrate by hydrogen sulphide. Any nickel present will be co-precipitated.	<b>Filtrate</b> . Acidify with acetic acid. A yellow precipitate of SnS <sub>2</sub> indicates <b>Tin</b> .
		I. Add potassium dichromate. A yellow precipitate of PbCrO <sub>4</sub> indicates <b>Lead</b> .	II. Add potassium ferrocyanide. A brownish precipitate or coloration indicates <b>Copper</b> .

Bigelow and Munson (*J. Amer. Chem. Soc.*, 1900, 22, 32) have modified Allen's original method, employing sulphuric and nitric acids followed by magnesium oxide and ignition. Later more nitric and considerable dilute hydrochloric acid are employed. The tin is precipitated as the sulphide along with lead and copper, from which it is later separated.

Schryver (*Report No. 7, Local Gov't. Board (Medical Department) of Great Britain*) destroys the organic matter by heating with potassium sulphate and sulphuric acid, as in the Kjeldahl method for nitrogen. Tin is removed as the sulphide. When large amounts are present, it is weighed as the oxide, but when present in minute amounts, a volumetric method is used. The method makes use of the colour produced by tin on dinitrodiphenylamine sulphoxide. Schryver obtained the following results using his methods.

## TIN IN CANNED FOODS—SCHRYVER

Foodstuffs	Origin	Grains of tin per lb.
Bacon, sliced.....	U. S. A.	0.61
Beef essence.....	England (3 makers)	1.58 to 1.92
Beef extract.....	S. America (2 makers)	0.40 to 5.33
Curried rabbit.....	Australia	0.19
Fruits.....	{ London importer	0.33 to 1.03
	{ England	1.42 to 2.81
Jams.....	U. S. A. (tin pierced)	5.13
Lobsters.....	U. S. A.	2.39
Plum-pudding.....	England	trace
Pork-pie.....	England	2.92
Roast fowl.....	England	0.58 to 1.44
Salmon.....	British Columbia	0.4 to 0.6
Tomato soup.....	U. S. A.	3.5
Vegetables.....	Australia	1.51 to 2.19

Schreiber and Taber (*U. S. Dept. Agr. Bur. Chem., Circular 67, 1911*) criticise the methods previously proposed for the estimation of tin and propose a modified method for making a sulphuric acid digestion. To the meat, fish, or other food in a large Kjeldahl flask they add water, potassium sulphate and sulphuric acid with a few glass beads, and digest, adding more sulphuric acid and digesting for 6 hours. They have also proposed an alkali fusion method, using magnesium oxide, sodium hydroxide, sodium carbonate and alcohol. The details will not be given here.

Baker (*Orig. Communications, 8th Inter. Cong. Appl. Chem., 1912, 18, 35*) obtains tin sulphide in the usual way from 100 grm. of canned food (digesting with nitric and sulphuric acids and precipitating with hydrogen sulphide). This is then dissolved in hydrochloric acid with the addition of potassium chlorate; a few pieces of aluminum foil are then added to the boiling solution to eliminate all the chlorine, and the tin is reduced to the metallic state by adding about 1 grm. of aluminum foil, this and subsequent operations being carried out while an atmosphere of carbon dioxide is maintained over the surface of the liquid. The mixture is again heated, the tin dissolving to form stannous chloride and, after the addition of air-free water, the solution is titrated with *N*/100 iodine solution, using starch as indicator.

The methods adopted by the Association of Official Agricultural Chemists for metals in foods are applicable to canned meats (see *Methods of the A. O. A. C., 1925, p. 171*).

*Tin.*—For tin the following method of digestion is given.

Weigh 50–100 grm. of the sample, depending upon the quantity of dry substance present and the relative ease with which the organic matter is oxidised, into an 800 c.c. Kjeldahl flask and add 100 c.c. of strong nitric acid. Allow it to stand overnight (this procedure being preferred if much fat or sugar is present) or place the flask on a wire gauze over a free flame and heat until the contents boil quietly. Add 25–50 c.c. of concentrated sulphuric acid, depending upon the quantity of dry substance present in the sample, heat until white fumes are generated, cool somewhat, add 5–10 c.c. of strong nitric acid, and heat as before. Repeat the addition of nitric acid until the solution remains clear after nitric acid is boiled off and fumes of sulphur trioxide appear.

Add 200 c.c. of water to the digested sample and transfer to a 600 c.c. beaker. Rinse the Kjeldahl flask with three portions of boiling water, making a total volume of approximately 400 c.c. Cool, and add strong ammonium hydroxide until just alkaline, then 5 c.c. of strong hydrochloric acid or 5 c.c. of dilute sulphuric acid (1 + 3) for each 100 c.c. of solution. Place the beaker, covered, on a hot plate; heat to about 95°, and pass in a slow stream of hydrogen sulphide for an hour. Digest at 95° for an hour and allow the beaker to stand 1–2 hours longer.

Filter, wash the precipitate, dissolve the sulphide in ammonium polysulphide, reprecipitate by acidification, and later convert the sulphide into oxide and weigh.

Baker's volumetric method can be used in place of the gravimetric procedure.

Other metals may sometimes be present, but are of relative unimportance in canned meats.

### **Preservation of Meat by Low Temperatures**

**General Principles.**—Like the other methods of preservation, this one has been known and understood in its simpler aspects since the earliest times. In cold climates it is necessarily a thing which is forced upon the attention of the inhabitants, for example, by the freezing and consequent preservation of game. Taking advantage of climatic conditions and of natural ice and ice mixed with salt, meat has intentionally been preserved by low temperatures for hundreds or thousands of years.

The early knowledge of the subject of meats preserved by low temperature was of a general nature, fragmentary, and indefinite. During the last 25 years great additions to our knowledge of the subject have been made by several investigators.

For the purpose of considering the effects of low temperatures on meat, this may be considered as a material consisting of elongated cells or sacs containing in water solution proteins, extractives, and salts. Ice begins to form in such a solution when the temperature is lowered to  $-0.4^{\circ}$ .

The subject of preservation by low temperatures can best be considered with reference to two temperatures:

(1) The freezing-point of the meat juice ( $-0.4$  ( $31.3^{\circ}$  F.) for beef juice).

(2) The temperature at which meat is frozen solid—about  $-10^{\circ}$  ( $16^{\circ}$  F.).

In practice, chilled meat—beef, mutton, pork—is stored at temperatures ranging from  $0-3^{\circ}$  ( $32-38^{\circ}$  F.). Frozen meat is usually stored at about  $-9^{\circ}$ , although occasionally use is made of a higher temperature, and for certain foods, especially fish, a lower temperature is desirable.

When meat is stored, as it usually is, above its freezing-point at temperatures between  $0$  and  $3^{\circ}$ , all of the changes which usually occur at higher temperatures take place, but at a reduced rate. The chemical action of atmospheric oxygen on the fat (rancidity) goes on, but at a slow rate, especially as meat is usually stored in the dark. Enzymic action in the fat, which leads to the production of free fatty acids, is also extremely slow. The proteolytic enzyme of the muscle fibre is definitely active, and in 15-20 days produces a considerable effect on the meat, judged organoleptically. The action of bacteria is retarded but not stopped at these temperatures. Their colonies make definite progress into the meat in two ways: by uniform extension inward from the surface and by extension and locomotion along veins and arteries, nerve sheaths, etc. Penetration by the first method is uniform and slow; by the second method, irregular and more rapid (Richardson and Scherubel, *J. Ind. Eng. Chem.*, 1909, 1, 95). Large pieces of beef-sides and quarters can easily be kept in good condition for 30 days by this method of preservation, and are sometimes held by epicures for 60 days or even longer at the temperatures above mentioned ( $32-38^{\circ}$  F.). (See also p. 383.)

Meat solidly frozen represents a very different condition from that just described, inasmuch as in a solid medium the growth and reproduction of bacteria are prevented absolutely, the action of proteolytic enzymes is so slight as to be negligible and, at the temperature required, the oxidising action of the air on the fat goes on at a very slow rate. An examination of the effects of temperatures below the freezing-point upon meat discloses the following phenomena. With the reduction of the temperature ice begins to form at  $-0.4^{\circ}$  ( $31.3^{\circ}$  F.). In this reduction of the freezing-point the soluble proteins, since they are colloids, play little part. Strange to say, the ice forms outside the cells (or muscle fibres) instead of inside (Richardson and Scherubel, *J. Amer. Chem. Soc.*, 1908, **30**, 1545). As the temperature is lowered, more ice separates and a more concentrated solution remains within the fibres. The effect of this is to force the fibres to assume a smaller and smaller bulk, so that finally the fibres instead of lying adjacent to one another, as at higher temperatures, are shrunk to thin strands, and these are separated by relatively large ice masses. A cross section under the microscope shows the grouped and individual muscle fibres irregular in outline, and separated by relatively large ice areas. If the temperature is reduced sufficiently, the whole mass will be solid, but before this point is reached, the solution within the cells becomes so viscous that it is, for practical purposes, solid.

It should be emphasised that it is the solid condition in this (and other similar cases) and not any definite temperature which limits and determines bacterial growth and activity, for certain bacteria, in suitable liquid media, thrive down to a temperature at least as low as the cryohydric point of water-salt, namely,  $-22^{\circ}$ .

It has been suggested by Richardson that the term "cryabiatic point" be applied to the temperature at which any medium assumes a sufficiently solid or rigid condition so as to preclude bacterial growth and reproduction.

A convenient method for demonstrating the appearance of muscular tissue in the frozen condition consists in immersing sawed cubes, 1 cm. on an edge, in the frozen condition, in cold 95% alcohol. The alcohol extracts the ice and hardens the tissue in its frozen form and structure. The pieces can then be handled according to ordinary histological technique.



On the large scale chilled meats are held in "chill rooms" or "coolers" whose temperature is lowered by means of circulating salt brine cooled by means of boiling ammonia in a mechanical refrigerating plant. Frozen meats are held in "freezers" which are usually cooled by a direct-expansion ammonia system, but circulating calcium chloride brine may also be used. Formerly, large quantities of chilled beef were shipped from the United States of America to Europe, especially England, but since 1912 this trade has been almost nil, the current of trade being filled with frozen meat from Argentine.

Chilled and frozen meats are transported in refrigerator cars or in steamships provided with mechanical refrigerating plants.

The principal meats which are held in frozen condition are beef, and the parts of beef, mutton, poultry, and fish.

Recently, new methods of freezing meat and fish have been devised which employ thin slices or layers (about two inches thick) and very low temperatures, as low as  $-50^{\circ}$  F. Rapid transfer of heat is effected by metal (such as monel metal belts) through which, or against which, streams of brine at very low temperature (calcium chloride brine, usually) are directed. Some methods use atomised sprays of cold brine. The metal presses against the meat or fish on one or two sides of slabs or thin packages. The result is very rapid freezing, small ice crystals, and the formation of these crystals within the muscle fibers. There is no distortion or crushing of the cell and practically no drip on thawing. The meat keeps wonderfully if not allowed to thaw. Kept frozen and carefully wrapped and handled, such products may remain in essentially fresh condition for a long time. Unfortunately, the method is not applicable to thick cuts or carcasses of meat.

**Wholesomeness of Frozen Meats.**—During the past few years there has been much discussion on the question of the wholesomeness and nutritive value of frozen meats. After investigation it now appears that, under proper conditions, it is one of the best methods for the preservation of meats and poultry (Pennington, *Hearings before U. S. Committee on Manufactures Relative to Foods Held in Cold Storage*, 1911. Richardson, *loc. cit.* Richardson and Scherubel, *J. Amer. Chem. Soc.*, 1908, 30, 1515. Richardson, *Reports of First Congress of Refrigeration, Paris*, 1909. Pennington, *Circular No. 70, U. S. Dept. Agric. Bur. Chem.*, 1911).

Pennington believes that poultry can be kept in good condition frozen for 1 year. Experiments of Richardson indicate that both beef and poultry under suitable conditions can be kept solidly frozen with their wholesomeness and nutritive value unimpaired for more than 3 years and probably much longer. Decomposition of both fat and lean, if it occurs, takes place either before freezing or after thawing.

**Methods of Analysis for Frozen Meats.**—The general methods of analysis do not differ from those applicable to fresh meats. The histologic picture is altered so much by the older freezing processes and by the rate of thawing, that histological evidence in regard to chemical change in frozen meats may be of no value. Staining reactions, especially of nuclei and striae, are likely to be abnormal. The histological method in the hands of an experienced observer may afford evidence of the frozen condition in some cases, but not in all. Slow thawing tends to eliminate distortion and restore the tissues to the normal condition. New methods of freezing leave a normal appearance on thawing.

Maljean (*J. Pharm. Chim.*, 1892, 25, 348) bases a method for the detection of frozen meat upon an observation of G. Pouchet, that when fresh blood is frozen at 10° to 15° the corpuscles are ruptured and after thawing do not again appear in normal form, if indeed they can be observed at all. The method consists in expressing a drop of juice or blood (preferably from a blood-vessel) onto a glass slide and examining it through the microscope. The normal juice or blood shows corpuscles of normal form floating in a colorless serum, but that from frozen meat shows distorted corpuscles (or none at all), while the serum is dark. The observation is correct, but the method is difficult to apply, because it is often difficult or impossible to obtain juice or blood from chilled beef which shows corpuscles, especially where access to large pieces is not possible, and thus in any given case chilled meat might be mistaken for frozen. The method must, therefore, be used with circumspection.

**Chemical Composition of Frozen Beef.**—In the accompanying tables the results were obtained by using the general methods of analysis for fresh meats given on p. 280 *et seq.* The figures may be compared with the similar ones for fresh beef given on p. 219 and p. 221. All the estimations were made on beef "knuckles" (the crural

PART I.—ANALYSES OF FROZEN BEEF KNUCKLES. AGE, 33 TO 554 DAYS. TEMPERATURE OF FREEZER, 9-12° F. FIGURES ON BASIS OF ORIGINAL MEAT—RICHARDSON AND SCHERUBEL

How stored	Lab. No.	Killed	Ana-lysed	Age, days	Mois- ture %	Ash %	Fat spt. ext. %	Total N. %	Ann. Method I %	Ann. Method 2 %	Cold water extract						Meat base %	Acid as lactic %
											Total solids %	Ash %	Total N. %	Coag. N. %	Albu- nose N. %			
Open freezer.....	.....	10/12 1906	1/22 1907	102	77.02	1.20	1.80	3.64	0.030	.....	6.07	1.14	4.93	0.803	0.419	0.022	0.350	0.72
Open freezer.....	.....	10/12 1906	1/13 1908	458	75.74	1.23	2.12	3.42	0.024	0.012	5.59	0.96	4.53	0.733	0.361	0.023	0.337	0.92
Open freezer.....	.....	0/12 1906	1/13 1908	458	76.72	1.23	2.02	3.32	0.025	0.012	5.80	1.14	4.66	0.730	0.353	0.026	0.343	0.92
Open freezer.....	2935	10/12 1906	3/27 1908	529	76.59	1.24	1.73	3.50	.....	0.012	5.94	1.15	4.79	0.836	0.449	0.022	0.359	0.77
Open freezer.....	2926	10/12 1906	3/27 1908	529	75.32	1.29	2.13	3.61	0.029	0.013	6.27	1.09	5.18	0.861	0.468	0.021	0.360	0.74
Open freezer.....	3605	10/12 1906	4/15 1908	548	75.49	1.27	1.87	3.53	0.025	0.012	6.21	1.19	5.02	0.770	0.381	0.021	0.371	0.73
Open freezer.....	3606	10/12 1906	4/15 1908	548	76.83	1.25	1.05	3.47	0.024	0.012	5.98	1.18	4.80	0.828	0.458	0.025	0.379	0.66
Open freezer.....	3801	10/12 1906	4/21 1908	554	75.40	1.23	2.03	3.65	0.031	0.012	6.05	1.22	4.83	0.812	0.418	0.026	0.369	0.79
In hermetically sealed tinned pails.....	2546	2/11 1908	3/16 1908	33	76.76	1.20	1.17	3.31	0.026	0.009	5.56	1.16	4.40	0.766	0.373	0.023	0.359	0.72
In hermetically sealed tinned pails.....	3551	2/11 1908	4/14 1908	62	77.11	1.16	1.05	3.38	0.031	0.011	5.88	1.14	4.74	0.819	0.432	0.025	0.365	0.80
In closed glass jars.....	3735	8/20 1907	4/20 1908	212	76.66	1.22	1.31	3.65	0.030	0.010	5.98	1.09	4.89	0.795	0.420	0.021	0.350	0.88
In closed glass jars.....	3736	8/20 1907	4/20 1908	212	77.03	1.28	1.30	3.65	0.031	0.010	6.06	1.21	4.85	0.798	0.426	0.023	0.349	0.88
Maximum.....	.....	.....	.....	554	77.11	1.29	2.12	3.65	0.031	0.013	6.27	1.21	5.18	0.861	0.468	0.026	0.379	0.92
Minimum.....	.....	.....	.....	33	75.32	1.16	1.17	3.31	0.024	0.009	5.56	0.96	4.40	0.730	0.353	0.021	0.337	0.66
Average.....	.....	.....	.....	353	76.39	1.23	1.65	3.51	0.028	0.011	5.94	1.14	4.80	0.795	0.413	0.023	0.357	0.79

PART II.—ANALYSES OF FROZEN BEEF KNUCKLES. AGE, 33 TO 554 DAYS. TEMPERATURE OF FREEZER,  $9-12^{\circ}$  F. FIGURES CALCULATED TO MOISTURE, ASH, AND FAT-FREE BASIS—RICHARDSON AND SCHERUBEL

[illegible]

triceps of anatomists). The table on p. 379 is a recapitulation of the results obtained on frozen beef in comparison with fresh beef. The figures are essentially the same and show no progressive change of any kind.

Stiles (*Dept. Sci. and Ind. Research, Food Investigation Board, Spec. Report No. 7*, London, 1922) has made a study of the preservation of food by freezing, with special reference to fish and meat. He discusses the principles and theory of freezing and the physics and chemistry of the freezing process. The work is largely in the field of general physiology and is of interest to those wishing to have a better knowledge of the fundamental principles.

Wright (*Trans. Proc. New Zealand Inst.*, 1912, **45**, 1; *J. Soc. Chem. Ind.*, 1912, **31**, 965) reports his results of a study of the influence of cold storage on the composition of New Zealand lamb and mutton when exposed to temperatures from 2° to 19° F. for periods up to 160 days. Street (*Allen's Commercial Organic Analysis*, 4th Ed., Vol. 9, 613) has erroneously reported Wright's work as showing marked changes due to cold storage. As a matter of fact, the changes on the whole were slight. There was a loss of from 2.5 to 3.5 per cent of water with an increase of all other constituents of the meat, due to this drying out. The water extract showed an increase in soluble solids, total nitrogen, proteose, peptone, and meat bases, and a decrease in coagulable nitrogen. Ammoniacal nitrogen remained unchanged, while the total acidity increased somewhat. The detailed percentage results are shown in the table on p. 380. The methods of analysis were chiefly those of the A. O. A. C.

SUMMARY OF RESULTS OF ANALYSES OF FRESH BEEF KNUCKLES AND FROZEN BEEF KNUCKLES.  
ALL ANALYTICAL FIGURES ARE PERCENTAGES. ON BASIS OF ORIGINAL MEAT—RICHARDSON AND SCHERUBEL

Cold water extract																
No. samples	Age, days	Mois- ture	Ash	Fat	Total N.	Amm. N. Method I.	Amm. N. Method II.	Total solids	Ash	Organic extractives	Total N.	Coag. N.	Albu- mose N.	Meat base N.	Acid as lactic	
Fresh—Maximum.....	13	7	77.27	1.31	3.34	3.65	0.033	0.011	6.24	1.27	5.27	0.854	0.452	0.034	0.398	0.82
	Minimum.....	0	75.26	1.11	0.78	3.34	0.022	0.009	5.55	0.95	4.42	0.742	0.358	0.014	0.360	0.63
	Average.....	3.7	76.35	1.23	1.43	3.49	0.029	0.010	6.01	1.14	4.87	0.806	0.413	0.024	0.355	0.68
Frozen—Maximum.....	12	554	77.11	1.29	2.12	3.65	0.031	0.013	6.27	1.21	5.18	0.861	0.468	0.026	0.379	0.92
	Minimum.....	33	75.32	1.16	1.17	3.31	0.024	0.009	5.56	0.96	4.40	0.730	0.353	0.021	0.337	0.66
	Average.....	353	76.39	1.23	1.65	3.51	0.028	0.011	5.94	1.14	4.80	0.795	0.413	0.023	0.357	0.79

ON MOISTURE-, ASH- AND FAT-FREE BASIS

Cold water extract													
	No samples	Age, days	Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Organic extractives	Total N.	Coag. N.	Albumose N.	Meat base N.	Acid as lactic		
Fresh—Maximum.....	13	7	17.05	0.154	0.053	26.12	4.15	2.20	0.162	1.86	3.79		
	Minimum.....	0	16.22	0.107	0.043	21.51	3.56	1.01	0.069	1.61	2.63		
	Average.....	3.7	16.63	0.139	0.049	23.32	3.84	1.99	0.117	1.77	3.25		
Frozen—Maximum.....	12	554	18.22	0.154	0.061	24.67	4.09	2.20	0.130	1.82	4.59		
	Minimum.....	33	15.86	0.115	0.041	21.68	3.51	1.73	0.098	1.61	3.12		
	Average.....	353	16.93	0.133	0.049	23.20	3.93	1.99	0.111	1.73	3.83		

NITROGEN FIGURES AS PERCENTAGES OF THE TOTAL NITROGEN

Cold water extract									
	No. samples	Age, days	Amm. N. Method 1.	Amm. N. Method 2.	Total N.	Coag. N.	Albumose N.	Meat base N.	Acid as lactic
Fresh—Maximum.....	13	7	0.927	0.318	24.75	13.10	0.983	11.18	9.73
	Minimum.....	0	0.841	0.262	21.45	10.35	0.419	10.67	9.73
	Average.....	3.7	0.834	0.291	23.11	11.83	0.707	10.64	10.64
Frozen—Maximum.....	12	554	0.917	0.361	24.23	13.20	0.783	10.92	10.92
	Minimum.....	33	0.762	0.271	21.61	10.56	0.575	9.56	9.56
	Average.....	353	0.791	0.325	22.68	11.76	0.661	10.19	10.19

## EFFECT OF COLD STORAGE AT 2°-19° F. ON LAMB AND MUTTON—WRIGHT

Day	Water	Ash	Fat	Nitrogen	Solids	Ash	Organic extracts	Total nitrogen	Coagu- lable protein	Protease	Peptone	Meat bases	Ammonia	Acidity as lactic acid
Lamb														
0	73.68	1.08	5.29	3.205	5.18	0.91	4.27	0.708	2.22	0.26	0.07	0.83	0.031	0.54
14	72.70	1.12	4.52	3.545	5.70	0.96	4.74	0.772	2.10	0.35	0.17	1.01	0.032	0.62
28	73.30	1.12	4.74	3.525	5.86	0.94	4.92	0.812	1.99	0.38	0.30	1.12	0.034	0.62
60	72.54	1.17	5.82	3.366	5.68	0.90	4.78	0.789	1.80	0.36	0.26	1.17	0.032	0.60
90	70.24	1.14	4.86	3.839	6.52	1.08	5.44	0.898	2.02	0.40	0.33	1.33	0.034	0.60
120	70.36	1.17	4.92	3.818	6.24	1.06	5.18	0.856	1.93	0.38	0.29	1.29	0.034	0.66
160	70.08	1.16	5.04	3.866	6.58	1.09	5.49	0.907	2.05	0.41	0.35	1.33	0.038	0.66
Mutton														
0	71.49	1.04	7.00	3.198	5.16	0.80	4.36	0.742	2.40	0.35	0.09	0.81	0.036	0.61
14	71.68	1.04	5.27	3.552	5.63	0.85	4.78	0.812	2.24	0.42	0.17	1.03	0.034	0.65
28	70.64	1.16	5.42	3.602	5.80	0.82	4.98	0.848	2.18	0.44	0.24	1.12	0.038	0.66
60	69.26	1.21	6.38	3.710	6.13	0.91	5.22	0.887	2.14	0.48	0.26	1.23	0.038	0.65
90	69.86	1.20	4.98	3.895	6.62	1.02	5.60	0.952	2.11	0.53	0.34	1.40	0.036	0.69
120	68.98	1.23	5.24	3.986	6.79	1.01	5.78	0.983	2.09	0.55	0.44	1.47	0.039	0.74
160	69.66	1.18	6.02	3.779	6.52	0.94	5.58	0.948	2.16	0.50	0.31	1.38	0.037	0.68

Wright found peroxydase, catalase, and protase present in all the samples of lamb and mutton, but no invertase, lipase, or diastase.

The fat of the meats in cold storage showed the following changes:

ACIDITY OF FAT (AS OLEIC ACID)

Days in cold storage	Acidity of fat	
	Lamb %	Mutton %
0	0.22	0.26
14	0.24	0.26
28	0.24	0.28
60	0.24	0.26
90	0.28	0.28
120	0.26	0.28
160	0.28	0.30

Even after 160 days of cold storage the interior of the meat was free from bacterial infection. There was practically no change shown in the histological structure of these meats due to freezing and slow thawing. This is in contrast to the work of Richardson and Scherubel, and of Birdseye (see *Meat through the Microscope*, pp. 16-31) with beef, and may indicate that lamb and mutton may be more successfully frozen by older methods than can beef.

Ascoli and Silvestri (*Arch. d. farmacol. speriment. e Sci. affini*, 1912, **14**, 229) made a study of frozen Australian and Argentine beef that had been in cold storage about two months and compared this with fresh local (Italian) beef. They studied the chemical composition, digestibility, and histological and autolytic changes. The frozen meat showed a change in colour, an increase in soluble protein which exuded in the form of a reddish fluid when the meat was thawed, the development of a peculiar taste, and a decrease in the aromatic odour of the broth. The changes were ascribed to the action of enzymes, and were more apparent in the fat than in the muscular tissue. The authors conclude that frozen meats may be regarded as wholesome food and may be eaten without injury.

### Effect of Cold Storage above Freezing

Wright has made a study of the changes brought about in meat stored at room temperatures with and without the presence of



bacteria (*Trans. Proc. New Zealand Inst.*, 1912, 45, 1). The meats used were the same as those employed in the study just reported. The changes which occurred in the meat were followed by analysis

CHANGES IN MEAT DUE TO PUTREFACTION, RIPENING, AND  
AUTOLYSIS AS SHOWN BY COMPOSITION OF THE WATER  
EXTRACT—WRIGHT

	Total solids %	Ash %	Total nitro- gen %	Coag. pro- tein %	Pro- teose %	Pep- tones %	Meat bases %	Am- monia %
Lamb								
Fresh meat.....	5.18	0.91	0.708	2.22	0.26	0.07	0.85	0.031
14 days' bacterial action.....	9.12	1.12	2.461	2.83	0.68	0.24	0.34	2.12
7 days' ripening, no added bac- teria.....	6.24	1.01	0.884	2.45	0.53	0.14	1.01	0.075
7 days' autolysis, aseptic.....	5.72	1.02	0.764	1.81	0.38	0.21	1.09	0.038
Mutton								
Fresh meat.....	5.16	0.80	0.742	2.40	0.35	0.09	0.81	0.036
14 days' bacterial action.....	9.46	1.08	2.526	3.53	0.71	0.25	0.38	2.03
7 days' ripening.....	6.42	1.04	0.928	2.66	0.40	0.32	0.94	0.102
7 days' autolysis, aseptic.....	5.90	1.02	0.806	1.76	0.35	0.24	1.25	0.037

of the water extracts. To determine the changes due to putrefaction, samples of the meat were mixed with water and an infusion of putrefying meat and allowed to stand at room temperature. The extracts were analysed on days 2, 4, 7, and 14. The table shows the end result only. A second lot of the meats was treated as above with no added bacteria, but analyses of the extracts were made on days 1, 2, 3, 5, and 7. To still other samples a mixture of thymol and chloroform was added in order to prevent the action of all bacteria, and extracts were analysed on days 1, 2, 3, 5, and 7. The table shows only the end-results. The changes are progressively more extensive in the order of aseptic autolysis, ripening, and marked bacterial decomposition. This work, of course, throws no light upon the effect of storage at temperatures just above the freezing point upon the composition of meat.

The autolysis of beef and mutton has also been studied by Fearon and Foster (*Biochem. J.*, 1922, 16, 564) who removed the tissues from the animal immediately after death and separated the connective tissue, fat, etc. from the lean flesh. This was minced, mixed with four times its volume of water, a small quantity of toluene

was added, and the mixtures incubated at definite temperatures ( $37^{\circ}$ ,  $6^{\circ}$ , and  $0^{\circ}$ ). At regular intervals samples were removed, and total nitrogen and soluble nitrogen remaining after precipitation with metaphosphoric or trichloroacetic acids were estimated by the Kjeldahl method. When incubated at  $37^{\circ}$  the muscles showed an increase in the proportion of soluble nitrogen for about 8 days, afterwards remaining constant. At  $6^{\circ}$  and  $0^{\circ}$  autolysis was considerably retarded.

Richardson and Scherubel (*J. Ind. Eng. Chem.*, 1909, **1**, 95) used beef knuckles which were stored at  $+2^{\circ}$  to  $+4^{\circ}$  C. for periods varying from 7 to 121 days. Considerable changes in the composition of the samples were noted as the storage period was increased. Increases were noted in the percentage of total water-soluble solids, total soluble, coagulable, and meat-base nitrogen, but the increases were not regular. Evidences of bacterial action were apparent early in the investigation.

Emmet and Grindley (*J. Ind. Eng. Chem.*, 1909, **1**, 413) studied the changes that occurred in fresh beef stored at  $33^{\circ}$  to  $35^{\circ}$  F. They used the carcasses of two steers, analysing one half of each while fresh and storing the other half. One half in cold storage was removed at the end of 22 days, while the other remained for 43 days. The beef which had been stored 22 days showed distinct increases in the total soluble and the soluble inorganic phosphorus and a decrease in the non-nitrogenous extractives. Other changes were insignificant. The beef stored for 43 days showed greater chemical changes, but they were without appreciable effect upon the nutritive value of the meat.

A very extensive study is that of Hoagland, McBryde, and Powick (*U. S. Dept. Agr., Bull.* **433**, 1917) who review the work of Gautier, Richardson and Scherubel, Emmet and Grindley, Wright, and Ascoli and Silvestri.

Hoagland, McBryde, and Powick studied the changes which took place in fresh beef stored at temperatures above freezing, attempted to determine the causes of the changes, and ascertained the length of time that fresh beef could be held in such storage and remain in wholesome condition. In commercial practice beef is generally stored at  $34$ – $36^{\circ}$  F. for only long enough to chill the carcass thoroughly, and the meat is then distributed to the retail trade. The bulk of the meat reaches the retailer and consumer

before it has been in storage two weeks. Occasionally it is desired to ripen the meat specially by holding it in cold storage for two to six weeks. A dry cooler with good air circulation and a temperature of about 34° F. represents good American practice.

These authors, using a special technique, prepared beef samples aseptically and studied the autolytic changes. Only 24 out of 30 samples showed visible bacterial growth when held in sealed containers. Nine of the samples showed no visible contamination with bacteria after incubation periods of 7 to 100 days.

In analysing the various samples the methods of the A. O. A. C. were used in general. Extracts were prepared by means of 0.9% sodium chloride solutions saturated with thymol by shaking with a chloroform solution of the latter. One hundred grm. of the meat were macerated in a porcelain mortar with the salt solution, transferred to a 2,000 c.c. volumetric flask, and diluted to the mark. The extract was stored at 34°-36° F. with shaking 8 times during 24 hours. At the end of this time, the material in the flask was allowed to stand until the meat had well settled, and the liquid was then filtered off.

They report the composition of five different muscle bundles of a beef hindquarter stored for 48 hours at 32°-34° F. before dissection of the muscles and 24 hours thereafter. The water content varied from 74.95 to 75.89%, the fat from 2.21 to 1.34%, the ash from 1.07 to 1.10%, the total nitrogen from 3.51 to 3.32%, the ammoniacal nitrogen from 0.0093 to 0.0076%, and the total phosphorus from 0.210 to 0.204%. Of the materials extracted by the 0.9% salt solution, expressed as percentage of the original meat, the soluble nitrogen varied from 1.001 to 0.907%, the coagulable nitrogen from 0.551 to 0.453%, the proteose nitrogen from 0.029 to 0.016%, and the amino nitrogen from 0.091 to 0.086%.

Aseptic autolysis was followed in beef muscle by samples taken at random from the muscular tissue of a hindquarter of a steer carcass, analyses being made at 7, 14, 21, 28, 42, 64, 77, 93, and 100 days. The soluble solids and soluble nitrogen decreased at first and then increased. This, if due to *rigor mortis*, would indicate that *rigor* is not complete until after three weeks have elapsed. By the end of 100 days the water content had decreased to about 70%. The ammoniacal nitrogen increased from 0.0087% to 0.0629%. The soluble solids, soluble nitrogen, proteose nitrogen,

and amino nitrogen increased, whilst the coagulable nitrogen decreased. The acidity, expressed as lactic acid, increased from 0.72 to 1.27%. The physical changes in autolysis were not marked, but there was a slight softening and exudation.

In the experiments conducted at cold-storage temperatures just above freezing, the carcasses were prepared and chilled, and the hind quarters held for various lengths of time in cold storage. One-half was analysed while fresh, and the other half was stored to observe the change. A total of seven carcasses was used. Four had a small micrococcus, not generally distributed but here and there some distance below the surface, and in both the fresh and chilled beef. In the quarter held 177 days it was more generally distributed. One of the fresh quarters showed also a small Gram-positive bacillus. In spite of these facts, the authors believe that the changes observed were not due to the bacteria. Bacteria and moulds grew to some extent on the surface. Histological changes in the muscle tissue could not be detected. Carcasses were stored for 15, 30, 45, 56, 66, 76, and 179 days. Temperature and humidity of the coolers were recorded. The temperatures ran from 32° to 36° F., and the humidity from 70 to 80% of saturation.

A typical result is that obtained from the round of carcass No. 5 stored 76 days and 21 hours.

	Water %	Fat %	Ash %	Total nitrogen %	Ammono- niacal nitrogen %	Total phosphorus %
Fresh round . . . . .	73.15	3.15	1.08	3.45	0.0101	0.206
Stored 77 days . . . . .	71.27	3.30	1.11	3.58	0.0133	0.206

	Total solids %	Solu- ble ash %	Acid- ity as lactic acid %	Solu- ble nitro- gen %	Coagu- lable nitro- gen %	Pro- teose nitro- gen %	Amino nitro- gen %	Solu- ble phos- phorus %	Inor- ganic phos- phorus %
Water ex- tract { Fresh	6.72	0.86	0.76	0.90	0.467	0.022	0.094	0.149	0.103
{ Stored	7.13	0.63	0.88	1.00	0.401	0.066	0.199	0.163	0.144

At the beginning the free fatty acids (expressed as per cent. of oleic acid) in the external fat, kidney fat, and intermuscular fat were 0.20 to 0.40%; at the end they had risen to 8.04%, 10.86%, and 1.70%, respectively.

The principal effects of storage upon the physical characteristics of the beef were shrinkage in weight and a hardening and darkening of the exposed muscular and fatty tissues. The shrinkage varied from 2.15% for 10 days to 10% for 177 days. Mould was slight and did not become excessive, even in the case of the beef stored 177 days. The physical changes which occurred within 2 to 4 weeks were not marked and did not lower the market value of the product.

The principal effect upon the organoleptic properties was a marked increase in tenderness, but the extent of this change did not bear a direct relation to the length of the storage period. In fact, the increase in tenderness of the beef stored for 2-4 weeks was about as great as that of beef stored for much longer periods of time. On the whole, the judges testing the meat for flavour held that storage did not improve the flavour of the meat. As storage became longer, the meat took on what was designated as an "old" flavour and was considered in some cases to be less appetizing than the flavour of fresh meat. Although in a few instances exposed portions of the stored quarters of beef showed signs of deterioration, still the edible portions of these quarters would have been classed as wholesome. The authors ate liberally of the test steak cut from each quarter of beef and in no case did they suffer any ill effects.

Briefly summarised, the changes which took place in the chemical composition of the beef during storage consisted in a gradual and partial transformation of the more complex constituents of meat into simpler compounds. In general, the extent of the changes increased with the period of storage. The changes were very similar in nature to, but less in extent than, those that took place in lean beef during aseptic autolysis. These changes consisted chiefly in increases in acidity; in proteose, non-coagulable, amino, and ammoniacal nitrogen; and in soluble inorganic phosphorus. Decreases occurred in coagulable nitrogen and in soluble organic phosphorus. The external and kidney fats showed marked increases in acidity.

The authors point out that the ammoniacal nitrogen, even after long periods of storage, was present in small quantities. They do not consider it to be a good index of change, and the changes shown are not of practical significance.

The chemical changes were without appreciable effect upon either the nutritive value or the wholesomeness of the edible portions of

the product. However, the changes that took place in the kidney fat and external fatty tissue after the longer periods of storage rendered them unsuitable for human consumption. Such tissues had to be trimmed away if an edible product were desired.

While the beef was held in the experimental cooler for 177 days at temperatures above freezing, it was possible to hold beef in the cooler of a modern packing house for only 55 days. This was due to the much higher humidity of the packing house cooler—92 to 95% saturation.

Clifford (*Biochem. J.*, 1925, **19**, 998) studied the effect of short periods of cold storage on beef and mutton. She suspended the pieces of meat in large glass filtrate jars covered with clock glasses in order to avoid evaporation. The jars were stored at 25° F. and 35° F. Samples were analysed at intervals up to 13 days. The author concludes that up to the third day of storage the meats appear identical with freshly-killed meat. Under storage at 25° F. on the sixth day the meat stored at 25° F. showed ice spicules and the red colour of frozen meat. In hot English weather beef and mutton will not keep for 6 days in a room at 35° F. There is no change in total nitrogen, soluble non-protein nitrogen, amino nitrogen, carnosine, or creatine in meat kept at 35° F. for 3 days or at 25° F. for 13 days. If Miss Clifford had been as familiar with proper cold storage procedure as she is with hot English weather, she would have devised an experiment more fitted to test the effects of cold storage.

The latest publication of note to deal with the conditioning or ripening of beef is that of Moran and Smith (*Dept. Sci. Ind. Research, Food Investigation, Special Report No. 36*, London, 1929). They give an excellent description of the constitution of meat and present several first class photomicrographs. They describe *rigor mortis* and report some experiments in connection with this phenomenon. A good review is given of general trade practice as it is found in England. Some space is devoted to the cooling and setting of beef, to bacterial changes and chemical and physical changes during conditioning or ripening. Their account of the chemical changes rests entirely on the material contained in the report of Hoagland, McBryde, and Powick already reviewed. The third part of this publication deals with the palatability of beef, with methods of measuring palatability, and with the results of some experiments on the cooking of meats.

For some additional literature on refrigeration see: *Dept. of Sci. and Ind. Research, Food Investigation, Special Report No. 2*, London, 1919; *Meat through the Microscope*, Chicago, 1929; *Between Two Oceans, Rapid Chilling and Freezing Systems for Fish and Meat*, M. T. Zarotschenzeff, The Cold Storage and Produce Review, London, 1930.

### Preservation of Meat by Chemical Inhibitors

**Curing Agents and Antiseptics.**—Logic, convenience, and public policy require that a distinction be made between antiseptics and curing agents in a discussion of the preserving or curing of meats by chemical substances. Curing agents are such substances as salt, saltpeter, sugar, and vinegar, which are effective in restraining bacterial growth when used in fairly high concentration. Antiseptics, on the other hand, are generally much more powerful in their action on bacteria, are effective in low concentration, in most cases actually kill bacteria, and in all cases have no condimental or food value.

The condimental curing agents, such as salt, have long been in use for the preservation of meats. The agents of this class recognised by the regulations of the Bureau of Animal Industry include, salt, saltpetre, sodium nitrate, sodium nitrite, sugar, vinegar, wood smoke, and spices. The use of these substances for the curing of meat is allowed in the United States of America as well as in other countries. Salt is, perhaps, the most important item of the list.

The use of antiseptics, such as borax, boric acid, sulphurous acid and the sulphites, benzoic acid and the benzoates, appears to be a much more modern practice, having reached a maximum during the years 1890-1905. The use of these substances has since declined, chiefly because of the legal restrictions and agitation against their use resulting from the activities of such leaders as Dr. Harvey W. Wiley. Concerning the harmfulness or harmlessness of these products when used in small quantity much has been written and published (Liebreich's *Treatise on the Effects of Borax and Boric Acid on the Human System*. Wiley, *Bull.* 84, Pt. I, 1904, Pt. II, 1906, Pt. III, 1907, Pts. IV and V, 1908, *Bur. Chem., U. S. Dept. Agr. Reports of the Referee Board*, Ira Remsen, Chairman, *Bull.* 88, 1909, *Bull.* 94, 1911, *Bull.* 97, 1912, *U. S. Dept. Agr., Office of the Secretary*).

While views may differ as to the harmfulness of these products, it appears to the writer that but one public policy can be approved in the case of meats. That policy is an uncompromising attitude against their use, the argument to rest largely on the lack of necessity for their use when meats are handled properly and the encouraging of careless practices if the antiseptics are permitted. The rather wide use of adequate modern refrigeration has removed the last ground from under the advocates of the use of antiseptics in meats. The last to go in the United States are borax and boric acid, formerly used on the surface of cured meats for export shipment.

**Methods of Curing Meat.**—The methods of curing meat differ considerably, depending upon whether the meat is in large pieces, such as hams, shoulders, and bacon strips, or is comminuted, such as sausage. Some meats are between these two in size, such as canned corned beef, luncheon loaves, and other specialties. The curing agents are applied to the surface of the cuts of meat, or some may be pumped in solution into the meat; while in the case of the hashed meats, the ingredients, excepting the smoke, are mixed with the meat itself. There are several classes or methods of curing meat in use, and each produces a product that is characteristic. The types of product produced are: (1) dry-salt cured, (2) pickled, (3) sweet pickled, (4) box cured, (5) corned beef, (6) dried beef. Meats prepared by any one of the first four methods may be smoked. They are then classed as smoked meats. Dried beef is cured, smoked, and dried.

**Dry Salt Cure.**—The simplest method of curing meat is by the process known as dry curing, in which salt, or a mixture of salt, sugar, and saltpetre, sodium nitrate, or sodium nitrite (the last two alone or mixed) is sprinkled or rubbed on the surface of pieces which are then laid in piles, in chill rooms where the temperature is held at a little above the freezing point. The recommendations as regards temperature vary somewhat, one authority giving 2–3° C. (36°–38° F.), while another states that 38° F. is best, with a range of 35–40° F. The green meats may be dipped in strong brine to moisten the surface, or may be pumped with pickle solution which also moistens the surface, the object of the moistening being to make the dry cure stick well. The curing materials penetrate the meat by diffusion and after the lapse of a length of time, which varies with the thickness of the piece and other factors, enough of them will



have reached the centre, so that meat is judged to be "cured"—that is, preserved so that it can be removed to warmer storage or to the smoke house without danger of spoilage. This method of curing is applied to backs and sides of pork and to lesser degree to pork hams and bellies. The latter are usually smoked after they are cured, whilst the former are not. The following is a more or less complete list of products: bellies, jowls, Cumberlands, long clears, English backs, American backs, square shoulders, English bellies, and long cut hams. A modification of this method produces "dry," "fancy," or "box" cured bacon, the modification consisting in using salt, sugar, and saltpetre (or other colour-fixing agent) on the closely packed bacon strips or bellies placed in tight boxes.

*Curing in Brine.*—In this process the pieces of meat are immersed in a brine containing salt alone, or, as is customary in modern practice, salt, sugar and saltpetre, sodium nitrate or sodium nitrite. The brine is known as "pickle" and when it contains sugar it is called "sweet pickle," the meats cured by these processes being known as "pickled" or "sweet-pickled" meats, respectively. The method is applied to pork hams, shoulders, and bellies, various cuts of beef which go to make barrelled beef, dried beef, tongues, corned beef, and other products. Meats such as pork hams should have an inside temperature of  $34-36^{\circ}$  F. when going into pickle, and the curing cellar temperature should be  $36^{\circ}-38^{\circ}$  F. Higher temperatures may lead to partial spoilage, and lower temperatures retard the cure. Mild cured hams are left in the sweet pickle about  $4\frac{1}{2}$  days to the pound. Various factors, such as the strength of the curing solution, the strength of the pumping pickle, and the amount and distribution of the pumping solution, affect this time in cure. Some plants cure 3 days to the pound, others  $3\frac{1}{2}$  days, and still others 4 days. Bellies may be left in cure  $2\frac{1}{2}$  days to the pound. When fully cured, meats are usually soaked in water in order to reduce the percentage of salt in the exterior portions of the meat. The meats may then be smoked and stored. During the soaking and smoking processes a partial equalisation of curing agents between the inner and outer layers of the meat takes place.

*Special Varieties of Hams.*—Westphalian hams, as cured in north-western Germany, are intended to be sliced and eaten raw. After they are cured by one of the methods just described, they are smoked over a bright hardwood fire, into which juniper berries and twigs

are constantly thrown, for a considerable period (8 days) in order to dry the meat. The fumes from the juniper gives a special piquant flavour to the product. So-called "Italian" hams are pressed, after curing, in order to flatten them, thickly sprinkled with ground black pepper, and then dried in large dry rooms after the manner of summer sausage, the temperature being held at 10° to 15° C. These hams are also eaten raw. Sometimes hams are aged for periods ranging up to 3 years, either in the air or buried in wood ashes, to develop special flavours. Hams of this sort are eaten raw as a relish.

*Curing of Sausage.*—For the curing of sausage the same materials are used as for the curing of other meats, but since they are mixed with the hashed or ground product, the curing proceeds very rapidly. The curing materials, spices, and any amylaceous materials (such as corn flour) are mixed with the ground or hashed mixture of meats and the resulting mixtures are allowed to cure in trucks or pans for a period varying from a few minutes to 3 days (summer sausage). It is then sold as sausage meat or stuffed into appropriate casings (the prepared intestines, bladder, or weasand from the ox, sheep, or swine) and sold raw or after cooking, smoking, or drying, or a combination of these as the case may be. Small pieces of meat are sometimes sprinkled with curing materials and packed in barrels so tightly that although diffusion and curing take place, no brine is formed. Such meat is usually intended for sausage manufacture at a later date.

For a more detailed discussion of certain meat-curing problems see McBryde, *A Bacteriological Study of Ham Souring*, U. S. Dept. Agr., Bur. An. Ind., Bull. 132, 1911; and Moulton, *Meat through the Microscope*, Chicago, 1929, p. 39 *et. seq.*

**Definition of Sausage.**—According to the Standards adopted by the Association of Official Agricultural Chemists in 1908, sausage is defined as follows:

"Sausage, sausage meat is a comminuted meat from neat cattle or swine, or a mixture of such meats, either fresh, salted, pickled, or smoked, with added salt and spices and with or without the addition of edible animal fats, blood, and sugar, or subsequent smoking. It contains no larger amount of water than the meats from which it is prepared contain when in their fresh condition, and if it bears a name descriptive of kind, composition, or origin, it corresponds

to such descriptive name. All animal tissues used as containers, such as casings, stomachs, etc. are clean and sound and impart to the contents no other substance than salt.

"Blood sausage is sausage to which has been added clean, fresh blood from neat cattle or swine in good health at the time of slaughter."

This definition takes no account of the addition of starch or flour to sausage—a very general and world-wide practice. In the United States of America, corn-flour is very commonly used, and such sausage is labelled, in compliance with the regulations governing meat inspection under the Act of Congress, approved June 30, 1906, "Sausage with Cereal."

More recent definitions for pork sausage and sausage meat are given on p. 211.

**Regulations Concerning Sausage.**—U. S. Government regulations now acknowledge the practice of using corn flour in certain sausages. The regulations (*U. S. Dept. Agr., Bur. An. Ind., Order 211*, Effective Nov. 1, 1922) provide that cereal, vegetable starch or vegetable flour, milk or its products may be used up to 3.5 per cent of the sausage. In unsmoked or uncooked sausage 3% of water may be added, while in cooked or smoked sausage the added water may be up to 10%. (Regulation 18, Section 6, paragraphs 4, 5, and 13, revised in *Announcement of August, 1925*.)

When the above materials, excepting water, are added in amounts up to the maximum allowance, the regulations require a suitably placed declaration such as "cereal added." (Regulation 16, Section 3, paragraph 1, revised in *Announcement of August, 1925*.)

The regulations also recognise the right to use the permitted food dyes to colour the casings of sausage by either dipping or cooking in water containing a suitable amount of the dye (Regulation 16, Section 3, paragraph 2, *Announcement, January, 1923*). In such cases the sausage must be suitably labelled "artificially coloured."

The permitted food colours, as listed in the *Announcement of October, 1922*, are as follows:

Red Shades  
56 Ponceau 3 R  
107 Amaranth  
517 Erythrosine  
Yellow Shades  
4 Naphthol Yellow S  
94 Tartrazine

Orange Shades  
85 Orange I  
Green Shades  
433 Guinea Green B  
435 Light Green S F Yellowish  
Blue Shade  
692 Indigo disulfoacid

Yellow A B (Benzeneazo- $\beta$ -naphthylamine)

Yellow O B (Ortho-tolueneazo- $\beta$ -naphthylamine)

Since that time additional colours have been approved.

April, 1927 Fast Green F C F

March, 1929 Ponceau S X and Sunset Yellow F C F

August, 1929 Brilliant Blue F C F

In the above list the numbers refer to colours as listed in A. G. Green's edition of Schultz-Julius *Systematic Survey of the Organic Colouring Matters*, published 1904.

**Varieties of Sausage.**—The varieties of sausage are numerous and are different in different countries.

*German Sausages.*—German sausages are extensively made from blood, liver, heart, brain, etc., and from fresh, dried, smoked, and salted meat. An addition of farinaceous material, usually in the form of flour or pea-meal, is very common.

The following description of the chief varieties of German sausage is an abstract of that of J. König:

*Blutwurst* or *Rothwurst* is made with hogs' blood, bacon, and pork, sometimes with the addition of heart or kidney, and either with or without flour. These sausages are similar to those known in England as "black pudding." They soon undergo decomposition.

*Mettwurst* (bologna or thick sausage) is made from pork and lard, with an addition (*inter alia*) of beef or horseflesh.

*Cervelatwurst* or brain-sausage is somewhat similar to the last. The Italian sausage *Salamiwurst* receives an addition of red wine. *Knackwurst* is a hard sausage, of a similar composition to cervelat, but the meat is previously cooked.

*Leberwurst* or liver-sausage is composed of liver, lung, kidney, skin, and lard or suet, with or without flour. Liver-sausages readily undergo change, and are then liable to occasion symptoms of irritant poisoning.

*Trüffelwurst* is made from meat, fat, and flour, with an admixture of truffles.

*Schwartenwurst*, *Sülzenwurst*, and *Magenwurst* are German sausages made from skin, stomach, etc., boiled soft, and mixed with unsalted bacon and a little blood.

*Bratwurst* is made from fresh raw pork and bacon, flavored with salt, pepper, and lemon-peel or cumin.

*Frankfort style*, *Frankfurter*, and *Vienna style*, *Wiener*, sausages are small, filled into sheep's gut, and composed of raw, slightly smoked pork, flavoured with salt, nitre, and pepper.

*Reiswurst* and *Grützwurst* are sausages commonly manufactured in North Germany from oat- or buckwheat meal, blood, soft-boiled skin, bacon, spices, etc.

*Erbswurst* is composed of beef-suet, bacon, peas, onions, spices, etc. Sausages of this kind, when of French manufacture, often contain much coarse meal and husk. Hence the woody fibre is high (4.3%) as compared with that in German pease-sausages (0.88 to 1.08%). G. Heppe examined three samples of *Erbswurst* which contained 7.32% of flesh constituents. F. Hofmann found in one sample a mere trace of animal proteins, while another contained 16.45 of total proteins, of which from 2 to 3% was of animal origin.

The following analyses also show the variation in the composition of pease-sausages:

Authority	Date	Water %	Nitrogenous matters %	Fat %	Starch, etc. %	Salts %
Ritter.....	1870	29.25	16.02	29.70	11.94	13.19
König.....	1884	11.00	19.65	15.52	41.05	11.88

The so-called blood- and liver-sausages often contain more flour than blood, liver, or flesh.

A specimen of cooked Lorraine sausage, examined by Allen, contained smoked meat, gristle, pea-meal, and onions, and gave the following figures on analysis: water, 46.04; fat, 25.67; proteins, 15.49; gristle, 3.65; starch, 4.00; and ash (sulphated), 6.36%.

Sausages which are intended to be kept should not contain milk, flour, bread, or brains, and little water, 35% or, better, 30% being the proper amount.

The analyses on p. 395 showing different varieties of German sausages are by J. König.

*English Sausages.*—English sausages are generally very different from those of German manufacture. As sold (with the exception of “polonies”), they are made of uncooked and unsmoked meat, and are intended to be cooked and eaten while quite fresh. The addition of dry bread or biscuit is very common, but by no means invariable.

	Water %	Nitrogenous matters %	Fat %	Carbo- hydrates %	Ash %	Sum of constit- uents %
Cervelatwurst (brain-sausage).....	37.37	17.64	39.76	.....	5.44	100.21
Mettwurst (bologna or thick sausage).....	20.76	27.31	39.77	5.10	6.95	99.89
Frankfurter Würstchen (Frankfort small sausages)	42.79	11.69	39.61	2.25	3.66	100.00
Blutwurst (black pudding), best quality.....	49.93	11.81	11.48	25.09	1.69	100.00
Blutwurst (black pudding), ordinary quality.....	63.61	9.93	8.87	15.83	1.76	100.00
Leberwurst (liver-sausage), best quality.....	48.70	15.93	26.33	6.38	2.66	100.00
Leberwurst (liver-sausage), third quality.....	47.58	10.87	14.43	20.71	2.87	96.46
Leberwurst, without flour...	35.89	16.13	45.51	.....	3.72	101.25
Sülzenwurst.....	41.50	23.10	22.80	.....	12.60	100.00
Knackwurst.....	58.60	22.80	11.40	.....	7.20	100.00
Erbswurst (German pease- sausage).....	6.53	15.46	37.94	31.38	8.69	100.00
Trüffelpwurst, best quality...	43.29	13.06	41.27	.....	2.41	100.03
Schinkenwurst (ham-sausage).....	46.87	12.87	24.43	12.52	3.31	100.00

The following analyses of sausages obtained from reputable dealers in Sheffield were made in A. H. Allen's laboratory:

Description of sausage	Price per pound	Water %	Fat %	Proteins %	Gristle, etc. %	Starch %	Ash %
Pork.....	9d.	54.90	21.04	12.28	0.67	1.05	3.52
"Cambridge" pork...	9d.	51.54	29.72	9.45	0.72	2.20	3.47
Mutton.....	1s.	55.58	30.51	1.89	3.11	3.90	2.50
German.....	8d.	46.54	17.87	16.38	1.13	15.00	4.47
Polony.....	10d.	45.57	32.66	17.26	0.54	2.30	2.80

In these analyses, a weight of 10 grm. was dried at 105° for the estimation of the water. The dried substance was then extracted with ether in a Soxhlet tube, the solution evaporated, and the residual fat weighed. The residue insoluble in ether was moistened with sulphuric and nitric acids, ignited, again moistened with sulphuric acid, re-ignited, and the sulphated ash weighed.

For the estimation of the gelatinoids, a weight of 20 grm. of the sausage was disintegrated by stirring it in a basin with cold

water, the excess of water drained off, and the fragments of gristle picked out with a pair of forceps with the aid of a lens. They were then washed in succession with methyl alcohol and with ether, dried at  $100^{\circ}$ , and weighed. The nitrogen contained in the gristle, etc., thus found, was then determined by Kjeldahl's process, and the amount deducted from the total nitrogen found by the same process in the original sausage. The difference was regarded as protein nitrogen, and multiplied by 6.3 to find the proportion of these compounds present.

The starch was determined by Mayrhofer's process (see below). No allowance was made for that derived from the pepper, or other spices. No wheat-starch could be observed by the microscope in the case of the first two samples. The dry bread used in the manufacture of sausages may be taken as containing 60% of starch.

A. W. Stokes, in a communication to A. H. Allen (see second edition, Vol. IV, page 281), stated that in 1894 he found that sausages which were being extensively sold on street-stalls in the east of London contained 10% of flesh, 20 of fat, and 70% of bread. On being placed in water they disintegrated, the meat sinking rapidly, so that a fairly good separation of the constituents could be effected by elutriation. No proceedings were taken against the vendors, owing to the absence of any legal or authoritative definition of a sausage.

*French Sausages.*—French sausages differ from those of English and American manufacture chiefly because they contain large amounts of horse flesh. To some extent, horse flesh is a recognised ingredient of sausage throughout the continent of Europe. According to Mitchell (*Flesh Foods*, page 128) the following are some varieties of French sausage.

"*Saucisses* consist of a skin of pig's intestine filled with raw or smoked minced flesh (usually pork), and seasoned with salt, pepper, pimento, etc. They are termed *saucisses longues* or *saucisses plates*, according to their form.

"*Saucissons* only differ from *saucisses* in being larger, more compact, and generally more highly seasoned. There are many varieties, such as *Saucissons de Lyons*, *Saucissons cru*, etc.

"*Cervelas* are large, short sausages containing salted and spiced flesh. They appear to be analogous to the English *saveloys*. According to L. Baillet, French sausages are not intended to be kept for

more than a few days. While still firm to the touch and sound, they gradually acquire on keeping a sharp but not unpleasant flavour, and are then termed *piqué* by the manufacturers. At a more advanced stage of alteration the exterior assumes an earthy tint, and is sometimes perceptibly moist to the hand, these changes being accompanied by the production of an acid and disagreeable odour. This condition is termed *échauffé*. Baillet states that in the east of France the addition of starch (up to 15%) is a common practice."

*American Sausages.*—In America, especially in the larger establishments, many different kinds of sausage are manufactured corresponding to German, Italian, Dutch, and other formulae. The following classification includes the principal kinds:

I. Domestic sausage. For prompt consumption.

a. Fresh pork sausage.

b. Cooked sausage:

Liver and blood sausage.

Head cheese and souse.

c. Smoked-cooked sausage.

1. Bologna sausage.

2. Frankfurter sausage.

II. Summer sausage. Well dried (moisture 30-40%). Perishability reduced to a minimum.

a. Smoked. Cervelats and German Salami, Farmer, Holsteiner, Goteburg, Roma, Mecklenburger, Mortadella, Capicola.

b. Unsmoked. Italian Salami, Milanese, D'Arles, Genoa, Lyon, Pepperoni.

III. Various cooked and smoked meats packed in sausage form, ready to be sliced and eaten. Various spicings and textures.

**The Water in Sausage.**—The less water there is in sausage the better will the product keep. To the total moisture content the lean meat contributes about 76% of its weight, fatty tissue 3 to 8%, starch and flour 10-15%. It is a very common practice to add water to sausage meat during the manufacture as an aid in stuffing the meat into delicate casings, and it is claimed that the use of excessive amounts of amylaceous substances is for the purpose of enabling the manufacturer to incorporate excessive amounts of water in his product. The moderate use of starch, flour, etc., (called "binders")



is for the purpose of causing the various ingredients to cohere, thus forming a mass of homogeneous texture. By various means known to sausage-makers lean meat can be made to absorb more than its normal content of water. It is, of course, obvious that water is not added to those sausages—such as the various summer sausages—which are intended for long keeping and which are dried.

The subject of water in sausage has been discussed at length elsewhere by the author (*Meat through the Microscope*, pp. 312-348). Some quotations from this work follow.

“Some of the materials that are used in the manufacture of sausage come from the less popular cuts of meat. They sometimes lose part of their water content through evaporation before being used for sausage. It is generally necessary to reduce the meat to a rather fine state before using it in sausages. This is done by means of rapidly moving knives or sharp blades in machines known as hashers or meat choppers. The friction of the knives passing rapidly through the meat causes heat which will not only tend to drive out some water but may encourage the growth of any bacteria which may be present. As a result the manufacturer generally adds crushed ice to the meat in the chopper. These conditions apply to the manufacture of sausage in general but are especially applicable to the sausages which are processed such as frankfurters, wieners, and bolognas. In addition, the public prefers its frankfurters to have a texture and consistency that can not be secured by the use of lean meats or meats containing medium amounts of fat unless a fair proportion of crushed ice is used. A succulent frankfurter is a fine food, but a dry frankfurter will soon drive the trade elsewhere. Bull beef has always been considered the best meat for frankfurters and bolognas. If it were used in the natural condition of moisture content it would make an entirely unacceptable article, but when mixed in the proportion of 60-100 parts of crushed ice to 100 parts of bull meat, it forms the basis of an excellent sausage. Furthermore, the processes of curing, smoking, cooking, and storing all affect the moisture content. During smoking and storing, water is lost. Unless this is allowed for in the manufacturing process, the sausage will be too dry.

“It has sometimes been stated that water is added to sausage for the purpose of adulteration and that it makes an inferior article. There may have been some reason for this statement, but it does

not adequately represent the facts. First-quality frankfurters are made from such materials as beef and pork trimmings and beef and pork cheeks. A fair proportion of ice in the chopper is required to give a good product. On the other hand, the second quality of frankfurters and bolognas contain tripe and weasand meat. These tissues are not of as high quality as the others and have a distinctly lower water holding capacity. This is due partly to the higher water to protein ratio which they exhibit and partly to the type of protein contained. Very much less water is added as crushed ice in the chopper to these second grade goods.

"For the foregoing reasons sausage manufacturers who are trying to approximate closely the water content favored by the government standard naturally experience great difficulty.

"There are at least eight factors which affect the water content of sausages. Some of these are within the control of the sausage maker and others at present seem not to be within his control. The two most important factors are: (1) the differences in composition of the various meats used in the manufacture of sausage, and (2) the addition of water in the form of ice at the chopper.

"The most easily controlled factor is the amount of ice added. If the manufacturer who is having trouble in keeping the water content of his sausage down, will reduce the added ice used, his trouble in this respect may partly disappear.

"Generally speaking, sausage manufacturers will find it helpful to know more about the composition of the raw materials they use. The following tables will be useful in showing how great these variations may be. Chemical analysis of the raw materials actually used by each manufacturer will help him in his problem.

"The manufacturer of sausage is interested in the moisture content of sausage for two reasons. First, the quality of the product depends, in a measure, on the water content. Second, there are definite and prescribed limits which food control officials have placed on the relative amount of water which sausage may contain."

Feder (*Z. Nahr. Genussm.*, 1913, **25**, 577; 1922, **43**, 193; *Chem. Ztg.*, 1914, **38**, 709), after analysing a large number of authentic samples of sausage, declared that the water content should not exceed 60%. He claimed that the ratio of water to fat-free organic matter is the most reliable index for a normal water content of tissues and should not exceed 4 to 1. This judgment has been con-

firmed by Schenck (*Z. Nahr. Genussm.*, 1915, **29**, 145). He found samples containing as much as 80% of water. König (*Chemie der Nahrungs und Genussmittel*, Vol. 2, 5th ed., Berlin, 1920) has gathered a fair amount of evidence to show that meat when freed of intermuscular fat and connective tissue has a remarkably constant water content, varying from 74 to 79%. Grossfeld (*Z. Nahr. Genussm.*, 1921, **42**, 173) and Pannwitz and Harder (*Z. Nahr. Genussm.*, 1922, **44**, 344) have also contributed data bearing on this question. Other investigations having a bearing on this question were discussed on pp. 248 to 251.

In applying the data concerning the water content of meat and of sausage to the inspection of sausage in the United States, the Bureau of Animal Industry determines the water content of the sausage and the total nitrogen. The latter is multiplied by the factor 6.25 to obtain the protein content. In fresh sausage the total water must not exceed 4 times the protein content by more than 3%, and in smoked or cooked sausage the total water must not exceed 4 times the protein content by more than 10%. Feder's original observation was rather more liberal than the Government's 4 to 1 ratio. In the latter the protein only is considered, while the original Feder rule counted all the fat-free organic matter, which would include glycogen, lactic acid, and similar substances.

The author has shown that, in the case of flesh or muscle tissue of cattle, hogs, and sheep, the water to protein ratio varies in some cases through wide limits, the extreme values being 2.1 and 7.7. On the average, the variation ran from 3 to 4 with most of the tissues, averaging 3.5 to 3.8 of water to 1 of protein. The water to protein ratio was very high in brine cured fatty tissues and low in dry cured salt pork. When the fatty tissues are rendered, water is driven off, and so the protein exceeds the water 3 to 10 times. In the so-called fancy meats such as brain, stomach, tripe, kidneys, tongues, and weasand meat, the water to protein ratio varied from 4:1 up to 8:1. Emaciation or extreme under-nutrition increase the ratio (see also Hoagland and Powick, *J. Agr. Research*, 1925, **31**, 1001) in the flesh, while in mature animals it is less than in very young animals. Sigler (*J. Assoc. Off. Agr. Chem.*, 1928, **11**, 112) presents considerable evidence bearing on this question. His and other data are discussed at length by the author elsewhere (*Meat through the Microscope*, pp. 312-348).

In this discussion the author presents data from a number of actual manufacturing operations with various kinds of sausage and shows the variations in water content that were found. In summarising he states:

"The variations shown by all tests reported certainly lead one to feel that the task of controlling the water content of sausage is a large one. Some of the variations may be due in part to errors in sampling or in chemical analysis, but this can not explain away much of the differences.

"Variations in the water to protein ratio in sausage materials and sausages may, then, be due to:

1. Differences between different classes of tissue.
2. Variations within the class due to age, emaciation, and fatness.
3. Differences due to sampling and mixing materials showing a lack of uniformity in composition.
4. Differences due to the curing process.
5. Differences in the practice of controlling added water.
6. Differences due to the smoking process.
7. Differences due to the cooking process.
8. Differences due to storage conditions.

"Control of the moisture content of sausage involves all of these factors. At least two of the factors, as has been pointed out, are more or less controllable. One can control the proportion of added water and one can take into consideration the water to protein ratio of different classes of tissue. The use of chemistry in analysing raw materials and finished products is desirable and helpful."

**The Function of Curing Agents.**—The chief function of the curing ingredients is to help prevent the growth of bacteria which have access to the meat. This is in contrast to the action of preservatives or antiseptics, since the latter act in low concentrations, while curing ingredients are only effective in relatively high concentrations. In food products generally both sugar and salt can be used to prevent the growth of bacteria. It is well known, for example, that salt meats keep well and that fruits preserved with sugar syrups keep from one season to another when sealed in jars. In meat curing, sugar, when used, is in rather low concentration. In other words, small proportions of sugar are used in sweet pickle formulas, and the proportions are not sufficient to act as a cure in the sense of preventing the growth of bacteria.

Perhaps the word "preventing" somewhat overstates the power of these ingredients with respect to bacteria. Some bacteria will grow in salt solutions and even survive on dirty rock salt. Others can grow in, or on, sugar solutions, and some, at least, can survive on dry sugar. Salt greatly discourages the growth of bacteria and in many cases actually prevents their growth where used in proper concentrations.

The chief function of sugar is to give flavour to the finished product. In low concentrations, or in dilute solutions, it is a food for numerous micro-organisms. It has sometimes been thought that the sweet pickle cure is a fermentation process in which bacteria take part. If such is the case, the sugar present would serve as food for these organisms while they are carrying on the process. There is another possible function of the sugar, and that is to encourage the growth of organisms causing fermentation and thus to discourage the growth of organisms causing spoilage or putrefaction. Certain bacteria can use more than one kind of food. When fed on protein, they may well cause a putrefaction, but when sugar is also offered as a food they will leave the protein alone and use the sugar. The evidence on all these points is not sufficient to answer the questions that have been raised. But there is some evidence, and this will be discussed later.

*The Function of Salt.*—Salt, sodium chloride, is the principal preserving material used in the curing of meats as commonly practised, but when used alone it does not produce the most satisfactory or best preserved meat. For the proper curing of meat not only salt, but sugar and saltpetre, sodium nitrate, or sodium nitrite are required, and also the use of low temperatures and finally smoking and drying. Salt appears to have little directly harmful effect on bacteria. In small quantity it is added to nearly all culture media. At higher concentrations it inhibits, but does not prevent the growth of bacteria, by increasing the osmotic pressure beyond that most favorable for bacterial growth. Some kinds of bacteria will not develop in salt solutions of greater concentrations than 10%, others thrive in a saturated solution, even at low temperatures, and the curing pickle from meat in process of cure always abounds with a great variety of bacteria. However, the chemical activities of these organisms must be greatly reduced by the combination of low temperature and salt, as otherwise the meat would spoil or "sour"

as the expression is, whereas the percentage of meat which spoils or sours in process of cure is very low. The effect of salt in lessening the vitality of bacteria can be shown by growing gelatin-liquefying bacteria in nutrient gelatin media containing salt; if the percentage of salt is high enough, growth is retarded and the liquefying power lost altogether. In estimating the effectiveness of salt as an antiseptic in cured meats, it should be calculated as a percentage of the moisture present. Thus in a ham whose lean showed by analysis, moisture 65% and salt 5.5%, the concentration of the salt in the water would be 8.46%. The total osmotic effect of the solution in the meat, however, would be due not only to the salt, but to all the soluble substances present.

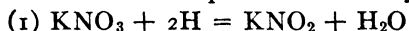
*The Function of Sugar.*—Sugar, sucrose, is used as a curing material primarily for the sake of the flavour which it contributes to the meat. It probably, also, exerts a slight beneficial effect in protecting the meat against proteoclastic decomposition by bacteria, in the same way that milk sugar protects casein in milk. The sucrose, by fermentation, raises the acidity of pickle and meat slightly.

*The Function of Saltpetre.*—Saltpetre performs several functions in the curing of meat. It maintains the red colour of the meat by the conversion of haemoglobin into a stable nitroso compound. This is brought about directly by the action of nitric oxide, which is formed by the action of nitrate-reducing bacteria on the saltpetre. It maintains aerobic conditions within the meat, thus tending to prevent the development of the typical anaerobic bacteria which cause putrefaction (Richardson *J. Amer. Chem. Soc.*, 1907, **29**, 1757–67). While it is not an antiseptic in the sense ordinarily understood, it appears to protect the nitrogenous tissue against bacterial attack, for meat cured without saltpetre produces many more sour pieces than meat cured with saltpetre (*Annual Report U. S. Bur. Animal Ind.*, 1908, p. 301). Sodium nitrate or nitrite is just as effective as potassium nitrate.

The maintenance of a red colour in meat through the reduction of saltpetre by bacteria involves several chemical changes. There are, in all probability, several red nitroso derivatives of haemoglobin, chief of which are nitrosohaemoglobin and nitrosohaemochromogen, any one of which can impart the red colour to meat. When no saltpetre is used in the curing of meat, the sodium chloride very quickly causes the red colour of fresh meat to disappear, and the

meat to turn dull gray. When saltpetre is used in the curing, this gray colour, sooner or later, depending on temperature and other conditions, gives place to the red colour of the nitroso derivatives. The nitric oxide which brings about this reaction is produced according to the following equations:

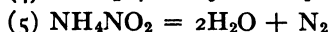
Reduction of saltpetre to nitrite by bacteria.



Reaction 2 is brought about by the lactic acid in meat resulting from *rigor mortis*. It is stated by some authorities that nitrates are reduced to nitrites by the direct action of meat (*Annual Report U. S. Bur. Animal Ind.*, 1908, 307; Abelous and Gérard, *Compt. rend.*, 1899, 129, [iii], 164-66). Richardson, working on perfectly fresh meat with careful technique, finds that meat does not bring about this reduction. Most samples of saltpetre give the test for nitrites with Griess's reagent. The surface of old meat is always covered with bacteria, and hashing the meat distributes them throughout the mass, and the reduction experiments have been made on hashed meat. One or both of these causes have probably contributed to the reports that meat alone reduces nitrate to nitrites. If meat reduced nitrates, the nitroso derivative could be produced by injection of a suitable nitrate into the meat, but this is not the case.

The experiments of Haldane (*Hygiene*, 1901, 1, 115), of Hoagland (*Annual Report U. S. Bur. Animal Ind.*, 1908, 301), and the experiments of Richardson, point to the following explanation in connection with the action of saltpetre in the curing of meats. When salted or pickled, the oxyhaemoglobin in a piece of meat is changed first to methaemoglobin, then in the interior to haemochromogen. The nitrate is reduced to nitrite chiefly in the pickle and, being produced in small quantity, penetrates the meat more slowly than the saltpetre, so that meat is often found whose centre contains only haemochromogen (which is converted into haematin on exposure to the air, and turns gray or drab), whereas the outer parts are coloured red by nitrosohaemoglobin. In the presence of a little acid, under reducing conditions (absence of air) nitrites always produce a bright red nitroso derivative when acting upon haemoglobin, haemochromogen, or haematin.

Nitrates and nitrites gradually disappear in pickle and cured meats, probably according to the equations already given and the following:



These reactions take place when a nitrite is in contact with ammonia or an amino compound at boiling temperature, or at ordinary temperatures under the influence of denitrifying bacteria. In summer sausage, after 60 days, usually no nitrates and only traces of nitrites are found. If more than a certain quantity of saltpetre (2–3 oz. per 100 pounds) is used in curing meat, especially in sausage, after a time the meat becomes pitted, porous, and spongy, owing to the gases formed, NO and N<sub>2</sub>. The odour and flavour of such meat is pungent. Some of the flavour of old hams and summer sausage, prized by epicures, is due to this cause.

The NO derivatives in cured meats often, but not invariably, yield a bright red solution when the meat is extracted with ether, or alcohol and ether. This colour might be mistaken for an artificial colouring matter in the examination of sausage for dyes, and is, therefore, important to the analyst. This colour dissolves in the fat present in sausage, colouring it red, but the colouring matters of some spices, notably pimento, have the same property. The nitroso derivative upon standing in ether solution turns dark brown. Addition of ammonia precipitates it as a red layer at the bottom. This colour is found usually in old summer sausage cured with a considerable amount of saltpetre.

In the United States sodium nitrate has almost entirely replaced saltpetre.

*The Use of Sodium Nitrite.*—Polenske (*Arb. kais. Gesundh.*, 1891, 7, 47; 1892, 9, 126) seems to have been the first to find nitrites in cured meat and curing pickle. Nothwang (*Arch. Hyg.*, 1892, 16, 122) confirmed his results. The function of saltpetre was established by Lehmann (*Sitzb. physikal. Med. Ges. Wurzburg*, 1899, 4, 57) and Kisskalt (*Arch. Hyg.*, 1899, 35, 11). Weller and Riegel (*Forschungb. Lebensmittel*, 1897, 4, 204) worked on the nature of the colouring principle formed. The work of Haldane and of Hoagland has already been discussed. Hermann (*Arch. anat. physiol. Wissen. Med.*, 1865, 469) had been one of the earliest to investigate the reactions of nitric oxide and oxygen-free haemoglobin. Other



investigators were Abelous and Gérard (*Compt. rend.*, 1899, **129**, 164) and Glage (*Die Konservierung der roten Fleischfarber*, Berlin, 1909) who wrote a practical book on curing. A little later several proprietary mixtures containing nitrite were put on the market. These included "nitrosin saltpetre" (Ostertag, *Handbuch der Fleischbeschau*, Stuttgart, 1913), "Aula" salt (*Zeit. Fleisch und Milchhyg.*, 1915, **26**, 49), and "Lebbin" salt (Lebbin, Swiss Patent 73,375). Auerbach and Ries (*Arb. Reichsgesundh.*, 1919, **51**, 532) used an improved method of nitrite determination and showed that nitrite-cured meats often contained an amount of nitrite far in excess of that found in nitrate-cured meats. Later, Pollak (*Z. angew. Chem.*, 1922, **35**, 229) showed that with careful control a nitrite cure was practicable and that less nitrite could be left in meat cured with nitrite than was usual with the nitrate cure. Auerbach and Ries (*Z. angew. Chem.*, 1922, **35**, 232) subsequently accepted these conclusions.

The use of sodium nitrite in curing meats has been studied extensively in the United States in the laboratories of the Institute of American Meat Packers and of the Bureau of Animal Industry. Lewis, Vose, and Lowry (*Ind. Eng. Chem.*, 1925, **17**, 1243) have reported some results of this work in the Institute, and Kerr, Marsh, Schroeder, and Boyer (*J. Agr. Research*, 1926, **33**, 541) have reported the work of the Bureau of Animal Industry. The author has presented some of the work of Lewis and his collaborators in detail in *Meat through the Microscope* (see pp. 43-67). This work shows, in general, that sodium nitrite can replace either sodium nitrate or saltpetre in curing, but only about one-tenth as much material is required. The work also shows that salt, sodium nitrate, and sodium nitrite, but especially the latter, function as inhibitors of bacterial growth (*Meat through the Microscope*, pp. 155-170).

**The Smoking and Drying of Meats.**—After large pieces of meat are cured they are soaked in water for different lengths of time, depending upon the size of the piece and the temperature, and are then smoked. Sausages are, of course, smoked directly. The smoking is accomplished by hanging the meat on racks in a chimney below which a slow fire, fed by hardwood and sawdust, is kept burning. The usual duration of the smoking is from 1 to 10 days. The smoke deposits certain creosotic or empyreumatic substances upon the surface of the meat, thus giving it a distinctive flavour, and

at the same time the surface is dried. The drying is rather more important than the smoking so far as the preservation of the meat is concerned, and is assisted in some cases by steam coils built into the smoke houses.

**Composition of Cured Meats.**—The composition of mild-cured and smoked pork hams handled according to customary American practice, and the analyses made according to the general methods of analysis given on page 280 *et seq.*, is shown in the following table. In order to make a comparison between the composition of fresh and cured meats it is very necessary to calculate to the moisture-free, ash-free and fat-free basis or for a still more accurate comparison, on a basis free from moisture, ash, curing materials and fat. It can readily be seen that the analyst must be prepared to find considerable variations from these figures in individual examples, owing to the use of more or less curing materials, differences in the time of curing, etc.

AVERAGE COMPOSITION OF MILD-CURED, SALTED, AND SMOKED PORK HAM—Compiled by Richardson

Section	Salt %			Sugar %			Saltpetre %		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
1	7.0	4.0	5.5	1.85	0.80	1.20	0.30	0.18	0.22
2	6.0	3.5	5.0	1.05	0.55	0.70	0.25	0.16	0.20
3	5.5	3.0	4.5	0.80	0.40	0.55	0.20	0.14	0.17
4	5.0	2.0	4.0	0.70	0.30	0.45	0.15	0.11	0.13
5 (Fat)	1.2	0.5	0.7	0.10	0.05	0.08	.....	.....	.....
Butt.....	7.0	4.0	6.0	.....	.....	.....	0.30	0.20	0.25
Whole of lean portion...	.....	.....	5.0	.....	.....	1.00	.....	.....	0.20

Moisture, lean portion. Max., 65; Min., 60. (Dried ham, 30.) Average 62%.

AVERAGE COMPOSITION OF MILD-CURED BACON

	Salt %			Sugar %			Saltpetre %		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Lean.....	12.0	6.0	9.0	2.5	1.70	1.90	0.60	0.30	0.4
Fat.....	1.8	0.7	1.0	0.08	0.04	0.05	0.10	0.05	0.07
Whole piece.	5.0	2.0	2.5	1.7	0.60	0.80	0.16	0.02	0.06
Moisture, whole piece	26	15	20	.....	.....	.....	.....	.....	.....

AVERAGE COMPOSITION OF CURED AND SMOKED DRIED BEEF HAMS<sup>1</sup>

	Salt %			Sugar %			Saltpetre %		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Outside. ....	13.5	10.0	11.5	1.20	0.75	1.00	0.40	0.20	0.30
Inside. ....	13.0	9.5	10.5	1.00	0.50	0.85	0.30	0.06	0.24
Whole. ....	13.3	10.0	11.0	1.10	0.60	0.95	0.35	0.20	0.30

Moisture, whole. Max., 55.0; min., 48.0; av., 51.0%.

<sup>1</sup> Three kinds are commonly cured in large establishments, the "knuckle," "outside," and "inside," all derived from the "round."

**Methods of Sampling Cured Meats.**—In general, the methods of sampling for meats on page 277 are applicable here, but for certain purposes the samples in the case of ham, bacon, and dried beef are selected from circumscribed areas and do not represent the whole piece. For factory control purposes, as well as for specific informa-

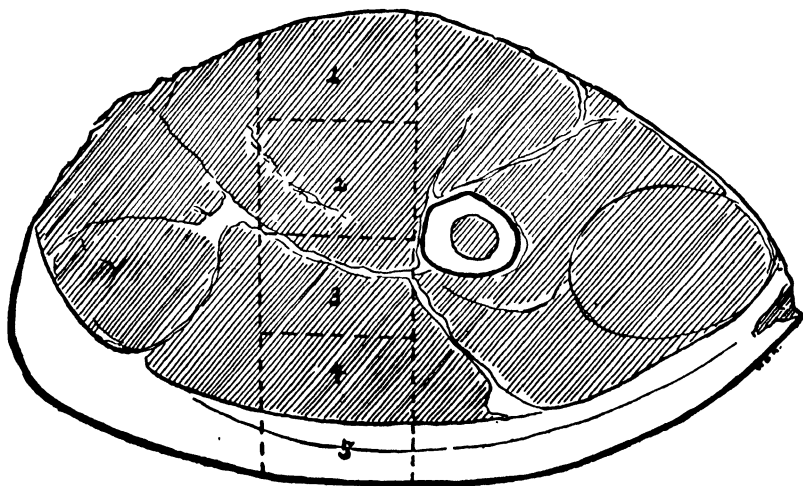


FIG. 22.—Method of sampling cured pork ham. Slice through thickest part.

tion, it is desirable to ascertain the distribution of curing materials, and for this purpose the following methods were devised.

**Sampling of Ham.**—If an average of the whole piece is desired, it is cut and sawed into parallel slices  $\frac{1}{2}$  in. thick, the bone, gristle, etc., separated from the lean, and the latter handled as in the case of fresh meats. To ascertain the distribution of salt, a slice about 1 in. thick is cut out of the thickest part of the ham and a strip removed,

as shown in the illustration. This strip is divided into 5 parts numbered from 1 to 5, and the salt in each of these estimated separately. Sometimes a sixth sample is taken from near the bone as shown, and a seventh from the opposite side. If it is desired to estimate the distribution of sugar and saltpetre, nitrate, or nitrite, as well as salt, parallel slices are cut from the thick part of the ham and a wider strip taken from each slice. Each strip is then sampled separately as described above, and the pieces bearing the same number combined for the estimations.

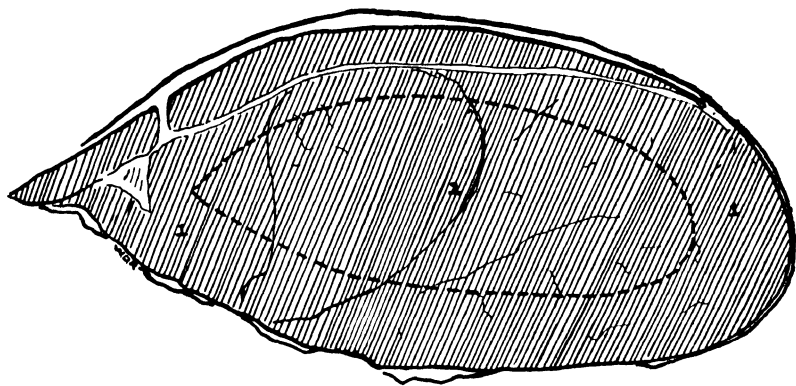


FIG. 23.—Method of sampling cured dried beef. (Cut shows an "outside" piece.)  
Slice through thickest part.

*Sampling of Bacon.*—Either the whole piece is taken for the sample and hashed, or the piece is cut into 1 in. slices and the lean dissected from the fat, each being ground and analysed separately.

*Sampling of Dried Beef.*—The pieces which are cured and smoked for dried beef are from the round and are designated as "outside," "inside," and "knuckle." The whole piece or a slice through the thickest part is usually analysed; but if it is desired to ascertain the distribution of curing materials, the piece is cut into parallel slices and a ring or band about 1 to 1½ inches wide is cut from the circumference of each slice and numbered 1. The interior is numbered 2. Samples bearing the same numbers from the different slices are combined.

*Sampling of Sausage.*—In sampling sausage it is sufficient to remove the casing and grind or hash the contents.

For the A. O. A. C. sampling methods see p. 279.

### Methods for the Analysis of Pickle and Cured Meats

**Analysis of Pickle.**—The specific gravity or salometer reading of a pickle gives very useful information concerning the concentration of salts and other materials in solution. In taking the salometer reading, cool the sample to 40° F. and take the salometer reading by means of a salt hydrometer. Such an instrument is generally provided with a scale reading from 0° to 120°. The specific gravity is determined at 60° F. by means of a specific gravity hydrometer. All aliquot parts for analysis are to be taken at 60° F. in order to keep the volume-weight relation constant.

If the pickle is new, it consists of water (and the salts naturally present in it), salt, sugar, and saltpetre, sodium nitrate or sodium nitrite. In mixed cures both nitrate and nitrite may be present. The salt can be determined by titration of the chlorine against silver nitrate, using potassium chromate as indicator. If the sugar used is a high-grade white or brown sugar, it can be determined with sufficient accuracy by the polarimeter, although the salts present influence the reading to some extent. The saltpetre, nitrate, and nitrite can be determined by the methods given below.

If, however, the pickle has been used, it will contain, besides the substances mentioned, the various extractive matters of meat. In this case the pickle may be considered as a cold-water extract and analysed accordingly. The coagulable, albumose, and meat-base nitrogen may be estimated as described under water extracts.

**Nitrate (Including also Nitrites).**—(a) Since sodium nitrate has practically replaced saltpetre, the directions will be given for it. The Schloesing-Wagner method (Wiley, *Principles and Practice of Agricultural Analysis*, Vol. 2, p. 397) is standard and is, in essence, as follows. This method determines nitrites along with nitrates.

A flask of about 250 c.c. is provided with a rubber stopper with two holes. Through one of them is passed a small separating funnel (100 c.c.). The other has a delivery tube, the end of which is so bent as to pass easily under the mouth of the measuring burette and is covering with a piece of rubber tubing.

Fifty c.c. of saturated ferrous chloride solution and the same amount of 10% hydrochloric acid are placed in the flask. The ferrous chloride is made by dissolving nails in warm 20% actual HCl and is kept tightly stoppered. The contents of the flask are boiled until the air is driven off. The delivery tube is then placed under

the measuring burette which is filled with a 40% sodium hydroxide solution, and is supported in a large evaporating dish three-fourths full of the same solution.

Twenty-five c.c. of a sodium nitrate solution containing 5 grm. of C. P. sodium nitrate to the litre are placed in the separating funnel and, with continued boiling, allowed to pass drop by drop into the flask. When almost all has run out, the funnel is washed three times with 10 c.c. of 10% hydrochloric acid, each 10 c.c. being allowed to pass into the flask. When no more nitric oxide is evolved, the measuring burette is transferred to a large cylinder filled with water.

Next, 25 c.c. of the pickle to be examined are taken and placed in the separating funnel (50 c.c. of ferrous chloride solution and 50 c.c. 10% HCl are first placed in flask). The contents of the flask are boiled until all air is expelled, the delivery tube inserted under the measuring burette, and the pickle allowed to drop into the flask. The separating funnel is washed out three times with 10 c.c. of 10% hydrochloric acid, and the burette transferred to the cylinder. After allowing it to remain there for about 3 minutes, the volume of nitric oxide is read by bringing the level within and without the burette to the same point. This volume is compared with the volume given by the 25 c.c. of sodium nitrate solution, and the calculation made as follows:

Twenty-five c.c. of sodium nitrate solution = 0.125 grm. of sodium nitrate. 0.125, divided by volume of nitric oxide from 25 c.c. = amount of sodium nitrate that corresponds to 1 c.c. volume.

Per cent. sodium nitrate in pickle =

$$100 \left( \frac{\text{amount of NaNO}_3 = \text{to 1 c.c.} \times \text{vol. given by 25 c.c. pickle}}{\text{sp. gr.} \times 25} \right).$$

The nitrogen excluding nitrates is estimated by the Kjeldahl-Gunning method, using mercury (page 286), after removing nitrates by adding to 50 c.c. of the pickle 10 c.c. saturated solution of ferrous chloride and 10 c.c. strong hydrochloric acid and boiling a sufficient length of time. This is necessitated by the fact that the modified Kjeldahl method, to include nitrates, does not yield correct results in the presence of much salt.

The total nitrogen is estimated by taking the sum of the nitric-nitrogen and nitrogen exclusive of nitrates.

The A. O. A. C. method (*Official and Tentative Methods of Analysis*, 1925, p. 238) is the same as the above in principle and differs only in details.

(b) Another method in use in packing houses is as follows:

**Apparatus and Reagents**

(A) Kjeldahl Distillation Outfit.

(B) Sulphuric Acid—exactly tenth normal.

(C) Potassium Hydroxide.

(D) Sodium Hydroxide—36° Baume “(30%).”

(E) Congo Red Indicator—dissolve 1 gram of congo red, Gruebler, in 10 c.c. alcohol and 90 c.c. of water.

**Determination.**—Pipette 25 c.c. of pickle into a 650 c.c. Kjeldahl flask. Add 200 c.c. of distilled water, 5 grm. of powdered zinc, 1–2 grm. of ferrous sulphate, and 50 c.c. of sodium hydroxide (36° BÉ.). Connect with the distilling apparatus, distil, collect the distillate in the normal way in *N*/10 sulphuric acid, and titrate with standard alkali, using congo red indicator. The results are expressed as sodium nitrate in terms of per cent. and pounds per 100 gallons.

**Example.**

Specific gravity (60° F.) = 1.200

20.00 c.c. of *N*/10 sulphuric acid

5.00 c.c. of *N*/10 potassium hydroxide

1.00 c.c. of *N*/10 acid = 0.0085 grams of sodium nitrate

$(20 - 5) \times 0.0085 \times 4 \times 8.35 = 4.26$  pounds per 100 gallons of sodium nitrate

$(20 - 5) \times 0.0085 \times 4 \div 1.200 = 0.43\%$  sodium nitrate

(c) Lewis and Blake, of the Institute of American Meat Packers, have made a critical study of methods of analysis for pickles and cured meats. They criticise the official method for nitrates and point out one cannot boil 100 grm. of meat with 35–50 c.c. of water. Such a mixture is semi-solid and will scorch over a free flame or on a hot plate. Evaporation of the filtrate, which is supposed to be obtained in sufficient volume for the work, causes a loss of nitrite, which is present not only in nitrite- and mixed-cured meats, but is also in nitrate-cured meats. This loss is roughly proportional to the degree of evaporation or time of heating. The fluid is generally sufficiently acid to permit a loss. The temperature at which the extraction is carried out and the length of time of heating affect

the recovery of added nitrite. The use of mercuric chloride as a protein precipitating agent also has an unfavourable effect. They propose a new method of extraction which gives sufficient liquid for the determination of salt, nitrite, and nitrate. This method recovers about 99% of added salt, a little over 100% of added nitrate, and about 90% of added nitrite. The method of extraction is given under nitrate in cured meat (pp. 410 and 427).

The end-point in the evolution of the nitric oxide depends on the stopping of bubbles of gas issuing from the end of the tube under the nitrometer. Lewis and Blake claim that one cannot tell when either the air originally in the reduction flask or the nitric oxide formed by the reaction is all out of the flask. A more or less continuous stream of small bubbles rises from the end of the delivery tube, which increases with the size of the flame used, and is greater when caustic soda solution is used as the liquid than when water is used. These bubbles are driven out of the receiving liquid by the hot stream (steam and gas) being boiled over. Cooling coils are of little value, since steam must reach the end of the tube in order to drive out all nitric oxide.

The method of preparing the ferrous chloride solution leaves much undissolved iron, and hydrocarbons are formed from the cast iron used. These by distilling tend to obscure the end-point unless they are filtered off. An unknown amount of hydrochloric acid distils over.

Sugar and proteins seem to have an effect on the ferrous chloride solution. There is some loss of nitric or nitrous acids which are formed when the solution being tested drops on to the boiling acid in the flask. A separatory funnel with a long capillary tube as stem, which dips under the acid, prevents the formation of these acids.

Lewis and Blake recommend the use of a Lunge nitrometer, in which the gas is introduced at the top and so prevents any appreciable loss of nitric oxide by absorption in the water. Distilled water is used as the liquid in the nitrometer. This water has been freed of air and thus causes no error due to dissolved gas. A little caustic soda solution introduced into the nitrometer at the end washes out any carbon dioxide in the collected gas. Solid ferrous chloride, comparatively pure, is used in place of the impure solution, with improved results. The failure to recover all added nitrite or nitrate



by this method is believed to be due to solution of the nitric oxide gas in the water which distils over.

The proposed modified method is given in detail below.

I. *Apparatus Required*.—The apparatus required for this determination is as follows: 500 c.c. Kjeldahl flask; 50 c.c. cylindrical separatory funnel with stem of 2 mm. bore; 2-holed rubber stopper; 50 cm. capillary condenser; Lunge nitrometer; barometer; pipettes.

The stem of the separatory funnel should reach the full length of the Kjeldahl flask and turn sidewise a short distance. (It will facilitate the replacing of the rubber stopper if the end of the tube is bent sidewise just far enough to still permit the removal of the stopper.) The capillary condenser tube should have an inner bore of about 2 mm. and an outside diameter of about 5 mm. It is connected with the flask and the top of the nitrometer by suitable rubber tubing. "Stop-cock grease," containing rubber, is recommended for the steam-tight joints, and castor oil for the air-tight joints.

II. *Reagents Used*.—The reagents used are: standard solutions of sodium nitrate and of sodium nitrite, each containing 2.0000 grm. of the salt per litre and preserved with chloroform; pure solid ferrous chloride, anhydrous or hydrated ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ); dilute hydrochloric acid, one volume of concentrated acid to five of water; air-free distilled water, freshly boiled and held at about  $160^\circ \text{F}$ . in a wash-bottle; caustic soda solution, about one-tenth saturated; rosolic acid solution.

III. *The Determination*.—The nitrometer is filled with cold water recently distilled. One hundred grm. of anhydrous ferrous chloride or 160 grm. of the hydrated salt are roughly weighed out and introduced into the Kjeldahl flask, together with a few small glass beads. One hundred c.c. of the dilute acid are now added, the stem of the separatory funnel is filled with warm air-free water, the flask tightly stoppered and connected with the condenser.

The flask is now heated with a 3-inch Bunsen flame separated from the flask by an asbestos wire gauze. The contents of the flask should boil in about 5 minutes. At the end of another 2 minutes most of the air should be out of the flask, water and air now occupying about equal volumes in the tube of the condenser. During this interval 50.00 c.c. of the standard solution, or 10.00 c.c. or

more of the pickle to be tested, are introduced into the cylinder of the separatory funnel.

The condenser is now connected with the top of the nitrometer, with its stop-cock open. The side-arm is then lowered. As soon as no more gas seems to collect at the top of the nitrometer, the stop-cock is closed, the side-arm raised so that the water-level therein is about an inch above the stop-cock. The gas and a few drops of water are then let out into the cup at the top of the nitrometer. The stop-cock is now opened into the condenser and the side-arm emptied down to the rubber tube connecting it with the nitrometer. If no appreciable volume of gas has by this time collected in the nitrometer, the determination may be started.

The stop-cock of the separating funnel is opened part way. The entire volume of liquid to be tested should enter the flask in a minute or less (depending on its volume). The stop-cock is then closed (without admitting any air), and the cylinder washed down with about 1 c.c. of concentrated hydrochloric acid. This liquid is then quickly admitted to the flask and the washing repeated. Finally, warm air-free water is added, of such volume (about 15 c.c. for 10 c.c. of pickle, but none for 50 c.c. of the standard solutions or of pickle) that the volume of liquid in the flask at the beginning of the next determination will be about what it was at the start (130 c.c. if anhydrous ferrous chloride was used). This entire procedure requires about 2 minutes.

The gas collected in the nitrometer is now read. The readings are at first made every 3 minutes, then every 2 minutes, and finally, every minute until the volume becomes constant or shows a decrease, in no case extending beyond 20 minutes after the determination was started. Carbon dioxide, formed by the action of ferric chloride on sugar and protein in the pickle, may still be coming off at the end of that time, but the nitric oxide is all out of the flask.

The stop-cock of the nitrometer is now closed, the nitrometer disconnected from the condenser, the side-arm adjusted to the level of the liquid in the nitrometer, and the reading made. This reading may be omitted if no knowledge of the amount of carbon dioxide is desired, but it also tends to show whether the gas is at room temperature. About 5 c.c. of the dilute caustic soda solution, made light red with rosolic acid, are now admitted through the cup at the top of the nitrometer (with the side-arm slightly lowered). The

nitrometer is now twice inverted without touching that part of the tube which the gas normally occupies. The side-arm is again adjusted, and the volume of gas read until constant for about 1 minute. The temperature is read on a thermometer hanging near the apparatus and the barometric reading is taken. Two readings of the barometer a day are usually sufficient.

The nitrometer is now emptied (otherwise slight variations in the readings would be caused by variations in the degree of saturation of the water with nitric oxide), rinsed, and refilled with distilled water. In about 5 minutes after the end of one distillation, another may be started, the solution next to be tested having been placed in the cylinder of the separating funnel at any time after the wash-water of the previous determination was all in the flask. Ten or more determinations may be made with the same charge in the flask, the process being continued until the contents of the flask bump (due to the separation of sodium chloride from the pickle). The organic matter in the pickle keeps the iron reduced, so that at the end of the series of determinations it is all ferrous, as shown by its green color (after settling), and by the usual absence of carbon dioxide formation at the end of each determination. If much less ferrous chloride is used, however, both sugar and protein in the pickle reduce part of the nitrate and nitrite below the stage of nitric oxide (probably to ammonia), so that the readings are low.

IV. *Calculating the Results.*—The barometric reading and the temperature of the room are observed. The difference between the coefficient of expansion for mercury and that for the brass scale is 0.000162 per 1° C., which must be subtracted from the observed barometric reading. It is found more convenient to correct all gas volumes to the average temperature and barometric pressure of the laboratory in question rather than to correct all to standard conditions (0° C. and 760 mm. of mercury). The correction to average conditions is generally done by inspection. Thus, if 26° C. and 746 mm. are average conditions and the readings are at 31° C. and 740 mm., the value at 26° C. is obtained as follows:—The barometric pressure at 26° C. equals the observed barometric pressure  $\pm (0.000162 \times \text{the temperature difference} \times \text{the observed barometric reading})$ . For a temperature difference of 4° C. or less, the correction is negligible. For differences of 4° to 12° C. it is 1 mm. of mercury. In the case supposed above a rise of 5° C. requires a

decrease of 1 mm., making the barometric pressure 739 mm. at 26° C.

The volume of nitric oxide must be corrected for vapour pressure of water at the temperature of the laboratory. The following table gives the data for most temperatures recorded.

Temperature, ° C.	Aqueous pressure
15.....	12.7 mm. of mercury
16.....	13.5
17.....	14
18.....	15
19.....	16
20.....	18
21.....	19
22.....	20
23.....	21
24.....	22
25.....	24
26.....	25
27.....	27
28.....	28
29.....	30
30.....	32
31.....	34
32.....	36

The water pressure corresponding to the temperature read on the thermometer near the top of the nitrometer is subtracted from the corrected barometric pressure just obtained. Thus, for the above case,  $739 - 34 = 705$  mm. of mercury, the pressure corrected for water vapor at 31° C. If the gas collected at the above temperature and pressure was 15 c.c. in volume, the volume at 26° C. and 746 mm. would be  $15 \times \frac{705}{746} \times \frac{299}{304} = 13.94$  c.c. Here the partial pressure variation is about 5.5%, and the temperature variation is about 1.7%, a total of 7.2%.

The results obtained for total nitric oxide must be corrected for that due to any nitrite found. The nitric oxide equivalent of the nitrite present is subtracted from that found by this method, to give the nitric oxide from the nitrate. It is customary to express the results in parts per million. To convert this to pounds per 100 U. S. gallons, one divides the parts per million by 1,168. To convert parts per million of nitrite into c.c. of dry nitric oxide at 746 mm. and 26° C. multiply by 0.00036 and then by the number of c.c. of pickle used in the nitrate determination. This gives the correction due to nitric oxide from the nitrite.

To convert the nitrate to pounds per 100 U. S. gallons, multiply the c.c. of nitric oxide due to the nitrate by 2.979 and divide by the c.c. of pickle used in getting the above c.c. of nitric oxide.

Thus if 10 c.c. of pickle yielded 14.4 c.c. of dry nitric oxide at 26° C. and 746 mm., and the nitrite in the pickle was found to be 85 parts per million,  $85/1,198 = 0.071$  pounds sodium nitrite per 100 gallons. The nitric oxide due to the nitrite in the pickle used for the nitrate determination was  $0.00036 \times 85 \times 10 = 0.3$  c.c. Therefore,  $14.4 \text{ c.c.} - 0.3 \text{ c.c.} = 14.1 \text{ c.c.}$  due to nitrate. This gives,  $\frac{2.979 \times 14.1}{10} = 4.20$  pounds sodium nitrate per 100 gallons.

For used or second pickle, 100 grm. of anhydrous ferrous chloride and 100 c.c. of hydrochloric acid (1 part strong acid to 5 parts water) are used in order to prevent the reduction of nitrates and nitrites by the organic matter in the pickle. In this case the gases collected are treated with a caustic soda solution to remove any carbon dioxide which may have been formed in the reaction. Ten c.c. of concentrated hydrochloric acid are added as a wash water to the reduction flask after each determination with used pickle. This is to keep the contents of the flask of proper acid strength.

*Nitrites.*—(a) Weigh out 10 grm. of pickle into a 500 c.c. volumetric flask. If the pickle is red, add dilute acetic acid until the colour is discharged, and make up to volume. Into each of several Nessler tubes place 2 c.c. of each of the following solutions. (Griess-Ilosvay reagent:)

(1) 1 grm. of sulphanilic acid is dissolved by heating in 14.7 grm. of glacial acetic acid diluted with an equal amount of water. By the gradual addition of water this solution is made up to 315 c.c.

(2) 0.2 grm.  $\alpha$ -naphthylamine is dissolved by heating in 14.7 grm. of glacial acetic acid and mixed with twice this amount of water and then diluted gradually to 350 c.c.

After the reagent has been added to the Nessler tubes, from  $\frac{1}{10}$  to 2 c.c., or a suitable quantity, of the diluted pickle, is added to different tubes and compared with standards in the usual way.

The A. O. A. C. method calls for the use of 5 c.c. of saturated mercuric chloride solution to be added to the solution containing nitrite in order to remove proteins. This is used in place of the acetic acid.

(b) Another method frequently used is as follows:

*Apparatus and Reagents.*—Flask—Fit a 300 c.c. Florence flask with a one-hole rubber stopper carrying a cylindrical, separating funnel of about 30 c.c. capacity, open top, with the stem just projecting through the stopper.

Potassium iodide—10% solution of potassium iodide.

Dilute sulphuric acid—10% solution of sulphuric acid.

*N*/20 sodium thiosulphate—(12.4 grm. per litre).

*N*/10 potassium dichromate—(exactly 4.903 grm. of pure recrystallised potassium dichromate per litre).

*Starch Paste.*—Mix 1 grm. of potato starch with 200 c.c. of water. Add 6 grm. of boric acid and heat the solution in an autoclave at 15 pounds pressure for twenty minutes. This solution can be prepared in quantity and preserved indefinitely by putting it in 4-oz. bottles, plugging the mouth with cotton and covering with cheese-cloth.

*Standardisation of Sodium Thiosulphate.*—Place 20 c.c. of *N*/10 potassium dichromate, to which have been added 10 c.c. of the solution of potassium iodide, in a glass-stoppered flask. Add to this 5 c.c. of concentrated hydrochloric acid. Dilute with 100 c.c. of water, then titrate with *N*/10 sodium thiosulphate, very slowly, until the yellow colour of the liquid has almost disappeared. Add a few drops of the starch paste and, with constant shaking, continue titrating with *N*/10 sodium thiosulphate solution until the blue colour just disappears.

*Determination.*—Place 5 c.c. of potassium iodide solution, 5 c.c. of dilute sulphuric acid, and 50 c.c. of water in the 300 c.c. flask. Insert the rubber stopper carrying the separatory funnel into the neck of the flask. Heat the solution to boiling and boil for about one minute to drive out all the air. Remove the heat and close the stop-cock. Cool the flask by allowing cold water to run on it for two or three minutes. Then run 50 c.c. (this can be measured carefully in a graduated vessel) of the pickle into the flask through the separatory funnel, taking care not to let any air into the flask. Wash the sample adhering to the funnel into the flask with a small amount of freshly boiled and cooled distilled water. Add about 2 c.c. of starch paste through the funnel, and if a blue colour develops, titrate with *N*/20 sodium thiosulphate, adding the thiosulphate through the funnel and washing into the flask with freshly boiled and cooled distilled water.

No air must be admitted to the flask during the determination, and there must be no leaks in the apparatus. If there is any air in the flask, the determination is worthless. The results are expressed as sodium nitrite in terms of per cent and pounds per one hundred gallons.

*Note.*—If the pickle is dark red in colour, it may give no blue coloration upon addition of starch paste. In such cases 50 c.c. of a 20% phosphotungstic acid solution are measured into a 250 c.c. volumetric flask, the pickle added to the mark, and the two thoroughly mixed. Upon filtering, a clear colourless filtrate is obtained which can be titrated satisfactorily. Fifty c.c. of the filtrate are equivalent to 40 c.c. of the original pickle.

(c) Lewis and Blake, working in the laboratories of the Institute of American Meat Packers, have devised the following procedure:

I. *Apparatus Required.*—Several 1.00-c.c. pipettes, graduated to hundredths; 2-c.c. volumetric pipette; 5-c.c. pipette, graduated to tenths; Nessler tubes (the height of the 50-c.c. marks must be identical, with 1 mm.) 50 c.c.; colour comparator (analysts are not agreed upon the advantages or disadvantages of a colorimeter in reading the tubes. No study has been made of different types, and therefore no recommendation is made on this subject).

II. *Reagents Used.*—*a. Standard Nitrite Solution.*—Dissolve 1.000 gm. of C. P. (The best sodium nitrite usually runs about 98% pure. For most packing-house purposes this is within the allowable error of determining nitrites. If, however, greater accuracy is desired, the nitrite should be standardised by titration against potassium permanganate, which has, in turn, been standardised against certified sodium oxalate, or otherwise. Silver nitrite may also be used as a standard) sodium nitrite in nitrite-free (distilled water is usually free from nitrites, but its purity should be tested) water. Dilute to 1 litre and mix thoroughly; dilute 100.0 c.c. of this solution to 1 litre and again mix; dilute 10.00 c.c. of this solution to 1 litre and mix thoroughly. Add six drops of chloroform to each of these solutions. The final dilution is used as standard and contains 0.00100 mg. sodium nitrite per c.c., or 1 part per million. These solutions are comparatively stable, but should be checked at times.

*b. Nitrite Reagent.*—Boil 1.00 gm. of alpha-naphthylamine hydrochloride in not more than 200 c.c. of distilled water, cool, filter, make up to 200 c.c., add 200 c.c. of a solution containing 2.0

gram. of sulphanilic acid (dissolve in hot water, cool, filter if necessary), and then add 10.0 c.c. of concentrated hydrochloric acid. This solution is colourless or nearly so and keeps for a month or more. Two c.c. are used for each test, no further addition of acid being necessary. The colour develops readily and becomes nearly constant after ten to twenty minutes, depending on the amount of standard solution used. The colour should not be read, however, after one hour, especially in the presence of much nitrite.

III. *The Determination.*—Dilute 1.00 c.c. of the pickle to 500 c.c. Mix thoroughly and measure out 1.00 c.c. and 10.0 c.c. into Nessler tubes. Simultaneously measure out 1.00 and 2.00 c.c. of the standard nitrite solution into Nessler tubes. Fill these tubes to the mark (approximately) with nitrite-free water, add 2.0 c.c. of the nitrite reagent, and invert each tube three times. After not more than ten minutes prepare other standards in the neighborhood of that one already prepared which most nearly matches the unknown, differing from each other by 0.10 c.c. for small amounts of nitrite, and by 0.20 c.c. for larger amounts. Make the final match when the standards last prepared are about ten to twenty minutes old. Solutions requiring over 2 c.c. of the standard nitrite for colour-matching are too dark to read accurately at the full height of the Nessler tubes, whereas those requiring less than 0.3 c.c. are too faint.

IV. *Calculating the Results.*—The concentration (if parts per million by weight are desired, divide the results obtained by the specific gravity of the original pickle at the temperature of the laboratory) of sodium nitrite in the original pickle may be readily calculated by the following formula:

$$\text{Nitrite in pickle (p. p. m.)} = \frac{\text{No. c.c. of standard used}}{\text{No. c.c. of diluted pickle used}} \times \text{dilution of pickle.}$$

Thus, supposing 1.00 c.c. of the original pickle match 1.00 c.c. of the standard; since the standard contains 1 part per million of sodium nitrite, obviously the pickle contains 1 part per million. If, however, 1 c.c. of the pickle matches 5 c.c. of the standard, for example, the pickle contains 5 parts of sodium nitrite per million; or, using the formula,

$$\text{Nitrite in pickle} = \frac{5}{1} = 5 \text{ parts per million}$$



If, however, the pickle in the latter case had been diluted 1 c.c. to 500 c.c., the original pickle is evidently 500 times as strong; that is,

$$\text{Nitrite in pickle} = \frac{5}{1} \times 500 = 2,500 \text{ parts per million}$$

The following table indicates how the entire range of nitrite concentration from  $\frac{1}{50}$  to 1,000 parts per million can readily be covered by using only the original pickle and the 500 dilution, on the one hand, and only two initial standards, on the other (with a few subsequent standards for the exact match).

RANGE OF PICKLE AND STANDARD DILUTIONS IN COLOR COMPARISON

Dilution of pickle	Pickle used, c.c.	Standard (1 part per million), c.c.	Nitrite in original pickle (parts per million)
None	50	1	$\frac{1}{50}$
None	10	1	$\frac{1}{10}$
None	1	1	1 <sup>1</sup>
One to 500	50	1	10
One to 500	50	2	20
One to 500	10	1	50
One to 500	10	2	100
One to 500	1	1	500
One to 500	1	2	1,000

<sup>1</sup> In pickle containing several p. p. m. sodium nitrite (probably 3 and above), the nitrite soon bleaches the colour first produced.

**Salt, Sodium Chloride.**—If the pickle is sufficiently pure, it may be titrated, after dilution with water, against *N*/10 silver nitrate solution, using potassium chromate as indicator. Or the pickle may be evaporated to dryness and charred, the char extracted with water, and the extract titrated as above. The titration, however, may be carried out according to Volhard's method, using thiocyanate as indicator in the presence of nitric acid.

The following is a much-used procedure.

**Reagents.**—(A) Silver nitrate. A solution containing 14.5304 gm. of silver nitrate per litre.

(B) *Potassium Chromate Indicator.*—A solution containing 1 gm. in 10 c.c. of distilled water.

**Reagents. Standardisation of Silver Nitrate Solution.**—Weigh out exactly 0.1000 gm. of C. P. sodium chloride (Mallinckrodt) that has been dried for 5 hours at 100° C., into a 100 c.c. casserole. Dissolve in about 25 c.c. of distilled water, add 4 drops of potassium chromate

indicator, and titrate to a permanent flesh colour. Exactly 20 c.c. of the silver nitrate solution should be required.

*Determination.*—Pipette 10 c.c. of the pickle into a 200 c.c. volumetric flask and make up to the mark with distilled water. Take a 10 c.c. aliquot portion of this solution, add 4 drops of potassium chromate indicator, and titrate to a permanent flesh colour. Report as pounds of salt per 100 gallons of pickle, and as per cent. In the case of spent pickle use the method as outlined in A. O. A. C. methods (1926)—p. 44, par. 13, 14, and 15.

*Example.*

Specific gravity (60° F.) = 1.200

25.00 c.c. Standard silver nitrate

1.00 c.c. Standard silver nitrate = 0.00500

$25 \times 8.35 = 208$  pounds salt per 100 gallons pickle

$25 \div 1.200 = 20.83\%$

The number of c.c. of standard silver nitrate used, multiplied by 8.35, gives pounds per 100 gallons when the standard solutions and the samples are prepared according to the above directions.

*Sugar.*—Either Allihn's method or Pavy's method may be conveniently used for this estimation. The Pavy method is conducted as follows:

The sp. gr. of the solution is first determined, after which 100 c.c. of the pickle are transferred to a 200 c.c. volumetric flask. Ten c.c. of strong hydrochloric acid are now added and the sugar inverted by Clerget's method—heating to 68° in water, taking 15 minutes to reach this temperature. The flask is removed, and its contents cooled and made almost neutral to methyl orange with sodium hydroxide (150 grm. NaOH to 500 c.c. water). The sugar solution is now made up to volume, and the sugar estimated as follows:

*Solutions:*

(1) 34.65 grm. of crystallised copper sulphate in 500 c.c.

(2) 173 grm. of potassium sodium tartrate and 160 grm. of potassium hydroxide in 500 c.c.

120 c.c. of each of these solutions are placed in a 2,000 c.c. flask, 740 c.c. ammonia water (sp. gr. 0.90) added, made up to volume, and standardised as follows.

One grm. of pure sucrose is placed in a 200 c.c. volumetric flask, dissolved in 100 c.c. of water, 10 c.c. strong hydrochloric acid added,

and the sugar inverted by Clerget's method, after which the contents of the flask are made up to volume. A small boiling flask of 200–250 c.c. capacity is fitted with a two-hole rubber stopper. Through one of the holes is inserted the tip of a 25 c.c. burette, and through the other the upper end of a bent delivery tube, leading under water in a beaker and fitted with pinch-cock and Bunsen valve at the lower end. The burette of this sugar apparatus is filled with the pure invert sugar solution. One hundred c.c. of the Pavy copper solution is placed in the boiling flask and boiled until no more air bubbles rise through the water from the Bunsen valve at the end of the delivery tube. The flame is now removed, the pinch-cock closed, and the sugar solution added, a little at a time, until the blue colour of the copper solution is discharged. The number of c.c. of sugar solution, multiplied by 0.005, equals the value of 100 c.c. of the copper solution in terms of sucrose.

The same procedure is followed in estimating the sugar in the inverted pickle solution. Nearly the total amount required for decolorising the copper solution is added all at once, to avoid protracted boiling and loss of ammonia. The amount to be added can be estimated by a preliminary approximate titration. The copper solution will stand a total continuous boiling of 5 minutes. The calculation is made as follows:

Weight of sugar in 100 c.c. of pickle equals 200 times the sugar value of 100 c.c. of copper solution, divided by the c.c. of pickle solution required to decolorise the mixture.

$$\text{Sugar \%} = \frac{\text{wt. of sugar in 100 c.c. pickle}}{\text{sp. gr.}}$$

Another much used method is the following.

*Sugar: Gravimetric.*

*Reagents.*—Fehling's solution I—Dissolve 34.639 grm. of copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in water, dilute to 500 c.c. and filter to free from sediment.

II—Dissolve 173 grm. of Rochelle salt and 50 grm. of sodium hydroxide in water and dilute to 500 c.c. Filter before using.

*Preparation of Gooch Crucibles.*—Cover the bottom of the crucible with a circle of filter paper and build a mat of asbestos  $\frac{1}{4}$  inch thick. The asbestos is prepared as follows:

Digest the asbestos, which should be of amphibole variety, with dilute hydrochloric acid (1:3) for 2-3 days. Wash free from acid, digest for a similar period with 10% sodium hydroxide solution, and then treat for a few hours with hot alkaline tartrate solution (old alkaline tartrate solutions that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash the asbestos free from alkali, digest for several hours with dilute nitric acid (1:3) and, after washing free from acid, shake with water into a fine pulp. In preparing the Gooch crucible, make a film of asbestos  $\frac{1}{4}$  inch thick and wash thoroughly with water to remove fine particles of asbestos. If the precipitated cuprous oxide is to be weighed as such, wash the crucible with 10 c.c. of alcohol, then with 10 c.c. of ether; dry for 30 minutes at 100°, cool in a desiccator, and weigh.

*Method.*—Pipette 25 c.c. of the sample into a 250 c.c. volumetric flask. Add 6 c.c. of concentrated hydrochloric acid and let stand overnight, or invert as follows:

Place the flask in a water-bath at 80° and raise the temperature of the pickle to 67° in 3 minutes. Maintain a temperature between 67° and 70° for 7 minutes. Cool at once, neutralise with 20% caustic soda, and make up to volume. If necessary, filter to obtain a clear solution.

Pipette 25 c.c. of this solution into a 250 c.c. beaker containing 25 c.c. each of Fehling solution I and II and 25 c.c. of water. Cover with a watch glass, bring to boiling in five minutes, and boil for exactly five minutes. Let the beaker stand for  $1\frac{1}{2}$  minutes, then filter, with suction, through the prepared Gooch crucible and wash thoroughly with water at about 80°. Transfer the crucible to a cool filter flask and wash with 10 c.c. each of alcohol and ether. Dry at 105° for 30 minutes, cool, and weigh. Calculate the weight of invert sugar equivalent to the weight of copper oxide found, from Munson and Walker's table "A. O. A. C." 1926, p. 434, or Leach "*Food Inspection and Analysis*" 4th Ed., p. 623.

Crucibles may be used several times without removing the precipitate, the new precipitate being filtered over the old. Mats can be cleaned by washing with dilute nitric acid (1:1) to dissolve the copper oxide.

*Example.*—Sugar — 25 grm. in 250 c.c. — 25 c.c. used.  
0.2647 grm. cuprous oxide

0.2649 grm. cuprous oxide (duplicate)

0.2648 grm. cuprous oxide (average) = 0.1238 grm. dextrose (table)

$0.1238 \times 0.95 = 0.1177$  grm. of sucrose.

$0.1177 \times 40 = 4.708$  grm./100 c.c.

$4.71 \times 8.35 = 39.32$  lbs./100 gals.

**Boric Acid.**—Boric acid was at one time used as a curing agent in pickles, but its use has now been abandoned. It may be estimated by the following method based on that of Thompson (*Analyst*, 1893, 18, 184). The quantitative estimation should always be preceded by a qualitative test with turmeric paper.

Borates are calculated as boric acid. New pickle containing no organic matter (other than sugar) and no phosphates may be titrated directly.

The sp. gr. is first determined. Then 50 c.c. of pickle are measured into a 3 or 4-in. porcelain evaporating dish, 25–30 c.c. of a 5% barium hydroxide solution added, and the liquid evaporated to dryness on the steam-bath. The residue is ignited, ground with a small agate pestle in the same dish, and extracted with 30–50 c.c. of hot water. The solution is decanted through a filter into a 400 c.c. Erlenmeyer flask, and the extraction repeated several times. Finally the char is transferred to the filter and thoroughly washed with boiling water. The filtrate is made very slightly acid to methyl orange, boiled for 1 minute, cooled and neutralised with sodium hydroxide solution. One hundred c.c. of C. P. glycerin are now added, and the solution titrated against  $N/2$  sodium hydroxide, using phenolphthalein as indicator. A blank of 100 c.c. of glycerin is titrated and subtracted from the previous titration. Mannitol may be used in place of glycerin.

1 c.c.  $N/2$  NaOH = 0.04775 grm. borax.

1 c.c.  $N/2$  NaOH = 0.02525 grm. anhyd. borax.

1 c.c.  $N/2$  NaOH = 0.031 grm. cryst. boric acid.

Dodd has recently made an exhaustive study of the methods of determining boron compounds in foods. The original papers (*Analyst*, 1929, 54, 645, 715; 1930, 55, 23) should be consulted by those interested.

**Analysis of Cured Meats.**—The general methods for the analysis of meat are applicable to cured meats also, and, therefore, only methods of special application are given here.

*Nitrate (Including Also Nitrite). Method A.*

*Solutions: Ferrous Chloride Solution.*—Dissolve 400 grm. of nails, tacks, or other small pieces of iron in a 2-litre Florence flask with 1 litre of strong hydrochloric acid, excluding the air from the flask by means of a stopper equipped with a Bunsen valve. When the evolution of gas ceases, transfer, and keep the solution in completely-filled 50 c.c. of glass-stoppered bottles. Employ only freshly opened bottles of the reagent for the determination. A modification of this method of storage is to transfer the solution to a suitable bottle from which the liquid below the oxidised surface can be drawn off when needed. The author has not tried this latter procedure.

*Standard Sodium Nitrate Solution.*—Dissolve 2 grm. of sodium nitrate in 1 litre of recently boiled water. Determine nitric oxide in 50 c.c. of this solution (equivalent to 0.1 grm. of sodium nitrate) as directed under "Determination."

*Apparatus.*—Clamp a 500 c.c. Kjeldahl flask fitted with a two-holed stopper to an iron stand. Through one of the holes pass the stem of a 100–125 c.c. cylindrical separatory funnel having a glass stop-cock, and into the other fit a delivery tube leading downward at an angle from the flask into a trough containing a strong solution of commercial sodium hydroxide (1 + 1). Terminate the upper end of the delivery tube just below the stopper in the flask and place the lower end, which is slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under the surface of the strong sodium hydroxide solution in the trough, the exit being below the mouth of an inverted measuring tube (50 c.c. plain eudiometer), or below the tube of a condenser joined to a measuring tube and having a glass stop-cock between them, either form of apparatus filled with a strong solution of commercial sodium hydroxide. A single coil of tin tubing fitted into the trough and carrying a current of cold water greatly facilitates the determination.

*Determination.*—Extract 100 grm. of the sample by boiling 6 to 10 times with successive 35–50 c.c. portions of water (see comments of Lewis and Blake, pp. 412, 429, 433), decant the extracts through a muslin or paper filter into a casserole, and evaporate the combined extracts to a volume of about 50 c.c. Introduce 50 c.c. of the ferrous chloride solution and 50 c.c. of dilute hydrochloric acid (1 plus 2.5) into the Kjeldahl flask, close the stop-cock of

the funnel, move the end of the delivery tube so that the escaping air will not pass into the measuring tube, and boil the contents of the flask until the air is completely expelled. Place the exit end of the delivery tube beneath the measuring tube (or condenser fitted with a measuring tube) and boil the contents of the flask one minute longer to make certain that no air remains. Introduce the concentrated extract of the sample into the flask, a little at a time through the funnel, continually boiling the contents of the flask to force the nitric oxide gas into the measuring tube. Finally rinse the casserole and the funnel three or four times with 5-10 c.c. of recently boiled water, adding the rinsings to the contents of the evolution flask in the manner described above. When the evolution of gas ceases, cover the opening of the measuring tube with a porcelain crucible, using tongs, and carefully transfer the tube to a tall glass jar containing a strong solution of commercial sodium hydroxide (1 plus 1), kept at room temperature. The temperature of the surrounding caustic solution will soon (10-15 minutes) be imparted to the contents of the tube, and the volume of nitric oxide is read with the tube in such a position that the level of the solution within the tube coincides with the level outside. (In the case of the condenser, the gas is transferred to the measuring tube after evolution has ceased and measured there after 15 minutes by making the level inside the tube the same as that outside in a levelling bulb. The gas is then released. While the gas is coming to temperature in the measuring tube, another sample is being run into the evolution flask. The time of evolution is regulated to be complete in 10-15 minutes so that after the reading is made and gas released from the measuring tube, the next volume of gas can be transferred to it from the condenser immediately.) Calculate the percentages of nitrates and nitrites as sodium nitrate from the volume of nitric oxide obtained from the sample compared with the volume obtained from 0.1 gram. of sodium nitrate, both measured under identical conditions.

After the measuring tube has been removed, quickly insert another filled with a strong solution of commercial sodium hydroxide (1 plus 1) over the delivery tube and boil 1 minute longer to make sure that all the nitric oxide has been expelled. Run another 50 c.c. portion of the standard solution into the apparatus and repeat the determination. Then run the samples in the same manner, in each case making certain that all the nitric oxide gas has been expelled.

After six to eight determinations are made, excluding the two standards, finally run another standard. The three standards should agree within 0.5 c.c. on about 30–35 c.c. One-tenth gram of sodium nitrate should give 26.36 c.c. of nitric oxide at 0° and 700 mm. pressure. Report results as percentage of sodium nitrate.

In place of the above method of extraction, 100 gm. of the prepared sample may be weighed into a 10-inch porcelain evaporating dish, 250–300 c.c. of distilled water added (or just enough for easy ebullition) and boiled for 10 minutes. The solution is decanted through muslin (starch-free) if the meat is lean, or through wet filter paper if the meat contains much fat, into an evaporating dish. In the latter case the contents of the dish may be chilled before each decantation to solidify the fat. The meat is extracted in this way three times and is finally transferred to the muslin and squeezed. The combined extracts are concentrated to a volume of about 50 c.c., and the determination continued as above.

*Method B.*—Lewis and Blake propose the following method as having many advantages over the above.

I. *Apparatus Required.*—The apparatus required for this determination includes all that listed under the Lewis and Blake method for determining nitrate in pickle, together with the following additional articles: enamelled graduated cup of about 400 c.c. capacity, preferably counterpoised; several wide-necked 250 c.c. graduated Erlenmeyer flasks; large wide-stemmed ( $\frac{1}{2}$  inch) funnel to fit flasks; long stirring rod to fit funnel, and several smaller stirring rods; several 500 c.c. Erlenmeyer flasks; several four-inch funnels; folded filters, diameter 18.5 cm. or more; wash-bottles, for hot and cold water; bulb wash-bottle for concentrated hydrochloric acid; water-bath; nut-butter grinder. (This should be a machine which delivers nearly the entire sample fed into it in a thoroughly disintegrated form.)

II. *Reagents Used.*—The reagents used are the same as those used for the determination of nitrate in pickle.

III. *The Determination.*—*a.* Preparation of meat samples. One or more thick slices are cut across the sample of meat submitted for analysis, from the centre if only one slice is taken, or distributed if several are taken. The edible portion (fat and lean) of these slices is then weighed. Usually the lean alone is used for analysis, although the fat and lean are separated and weighed, so that the



results can be expressed in terms of lean meat or of the entire edible portion. The portion to be tested is then cut into strips, and fed into the grinder, maintaining a constant ratio of fat to lean. The ground sample should be mixed by hand until it appears homogeneous as to colour and then repassed through the grinder. If not used at once, it is placed in a sterile covered bottle and stored at about 33° F. until used. This storage should in no case exceed a few hours.

*b. Extraction of Nitrate and Nitrite.*—One hundred and twenty-five grm. of the prepared sample are weighed out in the tared cup. Cold water is then added until the level in the cup reaches about 225 c.c., and the mixture thoroughly stirred. The contents of the cup are now poured into the large-stemmed funnel clamped above a 250 c.c. Erlenmeyer flask. By means of the long stirring rod this mass is transferred to the flask. The cup, stirring rod, and funnel are then washed with cold water in such fashion that the contents rise approximately to the neck of the flask. The flask is then rotated (it may also be necessary to rotate the flask, by temporarily removing the funnel, just before the last of the meat enters it, in order to settle the contents) and placed in the water-bath, kept at 195° F. The meat should then be thoroughly stirred with a stirring rod at frequent intervals for the next 10 minutes, then occasionally for the next 15 minutes. After half an hour, the flask is removed from the bath, cooled in running water nearly to room temperature (15 minutes), with gentle stirring every 5 minutes. It is then made up to volume (ignoring the layer of liquid fat), stoppered, thoroughly shaken, and the contents poured at once into a fluted filter held by a dry (Erlenmeyer) flask. It may be necessary to tap the bottom of the flask while transferring the mixture to the filter.

The filtrate is clear, nearly colourless, and about 100 c.c. in volume, passing the filter in about 15 minutes. This filtrate may stand in the flask, closed by the filter, for several hours without apparent change. In case the filtration is slow or of too small a volume for all the tests desired, a 5-c.c. portion may be taken for the nitrite test (no separate extraction for nitrite being necessary) and a 10-c.c. portion for chlorides (if desired), the additional amount required for the nitrate test being then obtained by throwing the meat residue onto 3 or 4 thicknesses of cheese-cloth. In case the meat is known to contain more than 0.05% of sodium nitrate, only 50 c.c. of the extract need be used for the nitrate test.

*c. Evolution and Collection of Nitric Oxide.*—Using 75 c.c. of the filtrate, this procedure is virtually the same as in the determination of nitrates in pickle, the use of two 5-c.c. portions of concentrated hydrochloric acid as wash-water for each determination (by means of the bulb wash-bottle) being absolutely essential here. No further addition of wash-water is necessary. On the contrary, it is best to use a 4-inch Bunsen flame under the reduction flask when 75 c.c. of the filtrate is used, in order that the volume of liquid in the flask at the beginning of successive determinations may be kept constant.

*IV. Calculating the Results.*—The concentration of sodium nitrate in the filtrate is calculated in exactly the same way as in the analysis of pickle. The final result, however, is expressed in per cent. of the sample of meat analysed instead of pounds per 100 gal., as in the analysis of pickle. This variation may conveniently be made as follows:

1. Calculate the volume of dry nitric oxide under average laboratory conditions which is due to the nitrate present in 75 c.c. of the filtrate, as in the analysis of pickle.

2. This value, multiplied by 3.17, gives the nitric oxide corresponding to 250 c.c. of the mixture or 125 gm. of meat. Experiments made on the analysis of sodium chloride in meat indicate that, under the foregoing conditions, a negative correction of 1% must be made in the observed values for each 25 gm. of lean meat present, due to the volume of the impenetrable substances of the meat. For 125 gm. this would be 5%, thus lessening the volume in which soluble salts are dissolved from 250 to 238 c.c.  $238 \div 75 = 3.17$ .

3. Assuming that 0.1 gm. of sodium nitrate yields 28 c.c. of dry nitric oxide under average laboratory conditions, 1 c.c. of nitric oxide is equivalent to 0.00357 gm. of sodium nitrate.

4. Therefore,  $0.00357 \times \text{total c.c. nitric oxide} \times 0.8 = \% \text{ sodium nitrate in meat}$ . That is to say, gm. of sodium nitrate in 100 gm. of meat yield per cent. The decimal 0.8 reduces 125 gm. of meat to 100 gm.

*Nitrite.—Method a. Solutions:* (a) Sulphanilic Acid Solution.—Dissolve 1 gm. of sulphanilic acid in hot water, cool, and dilute to 100 c.c.

(b) Alpha-naphthylamine Hydrochloride Solution.—Boil 0.5 gm. of the salt with 100 c.c. of water, kept at constant temperature for 10 minutes.

(c) Standard Nitrite Solution.—Dissolve 4.46 gm. of silver nitrite in nitrite-free water, precipitate the silver with sodium chloride solution, dilute to 1 litre, mix, and allow to settle. Dilute 100 c.c. to 1 litre and then 10 c.c. of this solution to 1 litre, using in each case nitrite-free water. Each c.c. of the last solution is equivalent to 0.000002 gm. of sodium nitrite.

(d) Saturated mercuric chloride solution.

*Procedure.*—Weigh 5 gm. of the prepared sample into a 50 c.c. beaker, and add approximately 40 c.c. of nitrite-free water heated to a temperature of 80° C. Mix thoroughly by stirring with a glass rod, taking care to break up all lumps, and transfer to a 500 c.c. volumetric flask. Wash the beaker and rod thoroughly with successive portions of hot water, adding all washings to the flask. Add sufficient hot water to bring the contents of the flask to about 300 c.c., place the flask on a steam bath, and leave for 2 hours with occasional shaking. Add 5 c.c. of saturated mercuric chloride solution, mix, cool to room temperature, make up to the mark with nitrite-free water, and mix again. Filter, and determine nitrite nitrogen as follows:

Place 2 c.c. and 20 c.c. of the filtrate in 50 c.c. Nessler tubes, make up to the mark with nitrite-free water, treat with 1 or 2 drops of strong hydrochloric acid, add 1 c.c. of the sulphanilic acid, 1 c.c. of the alpha-naphthylamine hydrochloride solution, and thoroughly mix. Make standards up by using 0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 c.c. of the standard sodium nitrite solution in other Nessler tubes, making to the mark with nitrite-free water, and treating with the hydrochloric acid, sulphanilic acid, and alpha-naphthylamine hydrochloride solution in the same manner as the sample. Set aside all the tubes for 30 minutes and determine the quantity of nitrite by comparing the depth of pink colour in the known and unknown solutions. Report as parts of sodium nitrite per million as follows:

(a) 5 gm.—500 c.c. — 2 c.c. aliquot part: Equivalent c.c. of standard  $\times 100$  = parts per million of sodium nitrite. (Range: 0.2 to 2.5 c.c. = 20 — 250 p. p. m.)

(b) 5 gm.—500 c.c. — 20 c.c. aliquot part: Equivalent c.c. of standard  $\times 10$  = parts per million of sodium nitrite. (Range: 0.2 c.c. to 2.5 c.c. = 2 — 25 p. p. m.)

*Nitrite.—Method b.* Lewis and Blake recommend the following procedure.

I. *Apparatus Required*.—The apparatus required for this determination includes all that listed under the Lewis and Blake method for the determination of nitrite in pickle, together with the following additional articles: counterpoised lipped weighing dish, preferably metallic; wide-necked short-stemmed funnel; thin stirring rod; meat grinder (this should be a fine-cutting machine which delivers almost the entire sample fed into it) or disintegrator; several 500-c.c. volumetric flasks; several 500-c.c. Erlenmeyer flasks; several 4-inch funnels; folded filters, diameter 18.5 cm.

II. *Reagents Used*.—The reagents used are the same as those used for the determination of nitrite in pickle, together with saturated (8%) mercuric chloride.

III. *The Determination*.—*a*. Preparation of meat samples. One or more thick slices are cut across the sample of meat submitted for analysis, from the center if only one slice is taken, or distributed if several are taken. These slices are then weighed, boned and skinned, and the edible (fat and lean) portion, which alone is used for analysis, is re-weighed. The lean only is usually analysed, but it would seem best to express all results in terms of the entire edible portion. This portion should be cut for grinding and then fed into the meat grinder in approximately the relative proportions of fat and lean present in the whole sample. The sample so ground should then be further passed through the grinder twice, with hand mixing after each grinding, until the product looks homogeneous with respect to fat and lean. If not used at once, it is placed in a sterile covered bottle and stored at about 33° F. until needed. This storage should in no case exceed a few hours.

*b. Extraction of Nitrite*.—A portion of the prepared sample weighing 10.0 gm. is weighed in the tared vessel. This is covered with water previously heated to about 150° F. All lumps in the sample are then disintegrated with the stirring rod, and the whole mixture at once washed into a 500-c.c. flask with warm water, using the wide-necked funnel. Under these circumstances the material should not clog the stem of the funnel; but if it should, it may readily be re-opened with the thin stirring rod. Warm water is then added to the flask until the total volume is about 400 c.c. The flask is then rotated and placed on a steam bath for half an hour, care being taken that the temperature does not exceed 150° F. The flask is then removed from the steam bath, 7 c.c. of saturated mer-

curic chloride added, and the flask cooled under running water. When cold, the volume is made up to the mark, the flask thoroughly shaken, and the mixture poured onto a dry folded filter, draining into a dry (Erlenmeyer) flask. When a small amount has passed through the filter, the colorimetric test is made.

*c. The Colorimetric Test.*—This is made on the filtrate in exactly the same manner as in the analysis of pickle, but without further dilution.

IV. *Calculating the Results.*—The concentration of sodium nitrite in the filtrate is calculated in exactly the same way as in the analysis of pickle, giving parts per million. This result may be transferred directly to the meat by the following formula:

$$\text{Nitrite in meat (p. p. m.)} = \frac{\text{Nitrite in filtrate (p. p. m.)} \times \text{Total c.c. of mixture}}{\text{grams of meat used for extraction}}$$

The volume of the mixture<sup>1</sup> is 500 c.c. To illustrate the calculation, assume that 1.00 c.c. of the filtrate from a sample of ham matched 1.85 c.c. of the standard solution (containing one part per million of sodium nitrite). Then

$$\text{Nitrite in filtrate} = \frac{1.85}{1} = 1.85 \text{ p. p. m.}$$

$$\text{Nitrite in meat} = 1.85 \times \frac{500}{10} = 92 \text{ p. p. m.}$$

Combining these two formulas, we have

$$\text{Nitrite in meat} = \frac{\text{c.c. standard used}}{\text{c.c. filtrate used}} \times \frac{\text{total c.c. of mixture}}{\text{grams of meat used for extraction}} = \text{p. p. m.}$$

*Salt, Sodium Chloride.*—Fifteen to twenty grm. of the hashed, minced, or finely cut sample are weighed out into a 3-in. porcelain evaporating dish and dried in the oven. (For quick work the sample is incinerated directly.) The sample is charred, but is not heated

<sup>1</sup> This refers to the original mixture of meat and water made to volume after extraction. Experiments indicate that soluble salts are uniformly distributed within this mixture, so that no correction is made for the volume occupied by the sample of meat.

This expresses the concentration of sodium nitrite in the edible portion of the meat. In case it is desired to express the results in terms of the whole specimen submitted, the foregoing results should be multiplied by the fraction.

$$\frac{\text{Weight of sample analysed}}{\text{Total weight of specimen}}$$

This assumes that the nitrite in the bone and skin are negligible.

sufficiently to volatilise sodium chloride, ground with a small pestle in the same dish, and transferred with hot water to a 200 c.c. volumetric flask. The solution is cooled, made up to volume and an aliquot of 20 c.c. titrated against  $N/10$  silver nitrate, using potassium chromate as indicator. This method is sufficiently accurate for all practical purposes.

Callow (*Biochem. J.*, 1929, **23**, 648) has devised a method for the determination of chloride in animal tissues which is applicable to a sample as large as 50 grm. and is simple and reliable. Fifty grm. of the finely minced meat are placed in a 300 c.c. beaker and covered with distilled water. The beaker and contents are placed on a boiling water bath, stirring the contents to prevent large lumps forming during coagulation. After coagulation the beaker is heated over a flame until it boils for a little while and then the clear liquid is filtered into a flask. This extraction is repeated 5-6 times for fresh meat and 10-12 times for cured meat. In the first case the volume is made up to 1 litre, while with cured meat the volume is made up to 2 litres.

Measured volumes of the extract are transferred to boiling tubes together with an excess of standard silver nitrate solution. A volume of concentrated nitric acid equivalent to the volume of the mixture is added, and the tubes are heated in a boiling water-bath for  $1\frac{1}{2}$  hours to destroy the proteins. (In the case of fresh meat with a low concentration of salt, the solution must be concentrated by evaporating—*in vacuo*—250 c.c. to about 10 c.c.) When cool, distilled water is added to dilute the strongly acid solution. The silver chloride is filtered off and washed, and the excess of silver nitrate is estimated by Volhard's method. Completeness of the extraction was proved by the absence of any chloride in the incinerated residue, using a dull red heat and extracting the charred mass. Also, all added sodium chloride was recovered.

Callow states that the usual incineration methods result in some loss of chloride. He found the use of sodium sulphate and iron alum when employed to obtain protein-free solutions also gave low results. Christy and Robson's method (*Biochem. J.*, 1928, **22**, 571) of estimating the excess of silver nitrate by potassium iodide solution and potassium bi-iodate in the presence of starch was found not to be accurate; the blue colour is obscured and the end-point is not reached until considerable excess of potassium iodide has been added. This

is due, states Callow, to the potassium iodide decomposing much of the potassium bi-iodate and giving free iodine, which does not give a blue colour. Treadwell (Treadwell-Hall, *Analytical Chemistry*, Vol. 1, Qualitative, 5th English Edition, 1921, p. 325) has shown that solutions of iodine in water are incapable of giving a blue colour with starch except in the presence of soluble iodide.

*Sugar.*—(a) One hundred grm. of the prepared sample are weighed into a 10-in. porcelain evaporating dish, 250–300 c.c. distilled water added (or just enough for easy ebullition) and boiled for 10 minutes. The solution is decanted through muslin (starch-free) if the meat is lean, or through wet filter paper if the meat contains much fat, into an evaporating dish. In the latter case the contents of the dish may be chilled before each decantation to solidify the fat. The meat is extracted in this way three times and is finally transferred to the muslin and squeezed. The combined extracts are concentrated to about 400 c.c. and transferred to a 500 c.c. flask. Fifteen c.c. of lead subacetate solution (U. S. P.) are added, and the volume made up to the mark. The solution is filtered through a folded filter and 400 c.c. (two 200 c.c. flasks) of the filtrate concentrated to 40 or 50 c.c. A few drops of lead subacetate solution are now added, then 10 c.c. of a saturated solution of sodium sulphate. The solution is filtered through a 9 cm. filter into a 100–110 c.c. sugar flask, the precipitate well washed with small quantities of hot water, the flask cooled, made up to 100 c.c. and inverted by Clerget's method. The solution is cooled, neutralised, made up to 200 c.c. in a second volumetric flask and the sugar burette (see *Analysis of Pickle-sugar*) filled with the solution. From this point the analysis is conducted as for sugar in pickle. Polarisation of the sugar solution is interfered with by the salt and extractives present. Fresh and cured meats containing no added sugar usually reduce an amount of the Pavy solution equivalent to 0.07–0.10% sucrose.

(b) *Solution.*—Phosphotungstic acid solution.—Dissolve 20 grm. of phosphotungstic acid in water and dilute to 100 c.c.

*Determination.*—Weigh 100 grm. of the finely ground sample into a 600 c.c. beaker, add 200 c.c. of water, heat to boiling, and boil gently for 5 minutes. Stir the contents of the beaker frequently during this and subsequent extractions to prevent lumping. Remove the beaker from the flame, allow the insoluble matter to settle, and decant the clear liquid on an asbestos mat in a 4-inch funnel. Filter

with the aid of suction. Add 150 c.c. of hot water to the residue in the beaker, boil gently for 5 minutes, allow the mixture to settle, and decant the clear liquid as above. Repeat the operation, and finally transfer the contents of the beaker to the funnel, wash with 150–200 c.c. of hot water, and press the meat residue as dry as possible. Transfer the contents of the filter flask to an evaporating dish and evaporate on a steam bath to a volume of about 25 c.c., but not to dryness. Transfer the extract to a 100 c.c. volumetric flask, taking care that the volume of liquid does not exceed 60 c.c. Add 25–35 c.c. of the phosphotungstic acid solution, shake vigorously, leave for a few minutes for gas bubbles to rise to the surface, make up to volume, shake, and either filter or centrifuge. The use of a centrifuge is to be preferred, since thereby a larger volume of liquid is obtained. Test a portion of the filtrate with dry phosphotungstic acid for complete precipitation. If an appreciable precipitate forms, take an aliquot part of the filtrate, add 5–10 c.c. of the phosphotungstic acid solution, make up to volume, filter, and test the filtrate for complete precipitation.

Transfer 50 c.c. of the clarified extract to a 100 c.c. volumetric flask, add 5 c.c. of strong hydrochloric acid, and invert in the usual manner. Cool the solution, neutralise to litmus, cool, make up to volume, and filter. To the filtrate add sufficient dry powdered potassium chloride to precipitate the excess of phosphotungstic acid, filter, test the filtrate for complete precipitation, and determine the reducing sugar in the usual manner by the Munson and Walker Method (*Official A. O. A. C. Methods* (1925) para. 35, p. 190). Calculate the total sugar as sucrose. In the case of fresh meats the calculation should be to dextrose.

If an abnormal reduction is obtained when the clarified meat extract is boiled with Fehling's solution, *i.e.*, if the solution turns yellow, brown, green, or muddy in appearance, instead of reddish-blue, the determination should be discarded, since incomplete precipitation of the nitrogenous compounds, due to the use of insufficient phosphotungstic acid, is indicated.

*Starch.*—Starch may occur in sausage, “devilled” meat, chopped meat, and similar comminuted meats. Several methods of estimation have been proposed.

(a) *A. O. A. C. Method. Qualitative Test.*—Treat 5–6 grm. of the sample with boiling water for 2–3 minutes, cool the mixture, and



test the supernatant liquid with iodine solution (0.05 grm. of iodine and 0.2 grm. of potassium iodide in 15 c.c. of water). In interpreting this test it should be remembered that a small quantity of starch may be present as the result of the use of spices. If a marked reaction is given, however, it may be concluded that starch or flour has been added, and a quantitative determination should be made. The qualitative test may be replaced by a microscopic examination, which discloses not only the presence of added starch but also the variety used.

*Quantitative Test.*—Treat in a 200 c.c. beaker 10 grm. of the finely divided sample with 75 c.c. of an 8% solution of potassium hydroxide in 95% alcohol and heat on a steam-bath until all the meat is dissolved (30–45 minutes). Add an equal volume of 95% alcohol, cool, and allow to stand for at least an hour. Filter by suction through a thin layer of asbestos in a Gooch crucible. Wash twice with a warm 4% solution of potassium hydroxide in alcohol (50% by volume) and then twice with warm 50% alcohol. Discard the washings. Retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker and add 40 c.c. of water and 25 c.c. of concentrated sulphuric acid. Stir during the addition of acid and make sure that the acid comes in contact with all the precipitate. Allow the beaker to stand 5 minutes; add 40 c.c. of water; and heat just to boiling, stirring constantly. Transfer the solution to a 250 c.c. volumetric flask, add 2 c.c. of 20% phosphotungstic acid solution, allow the liquid to cool to room temperature, and make up to the mark with water. Filter through a starch-free filter paper; pipette 100 c.c. of the filtrate into a 200 c.c. volumetric flask; neutralise with 10% sodium hydroxide solution; make up to volume; and determine the dextrose present in a 50 c.c. portion of the filtrate by the Munson and Walker method (*J. Amer. Chem. Soc.*, 1906, **28**, 663; 1907, **29**, 541; *Methods of Analysis of the A. O. A. C.*, 1925, 190) titrating the cuprous oxide precipitate. After washing the precipitate, dissolving on the Gooch crucible in 5 c.c. of warm dilute nitric acid (1 + 1), filtering and washing, the collected solution and washings are boiled to expel red fumes; 5 c.c. of bromine water is added; and the bromine is boiled off. Remove from the heat and add a slight excess of strong ammonium hydroxide (about 7 c.c. are required). Again boil until the excess of ammonia is expelled, as

shown by a change of colour of the liquid and a partial precipitation. Then add a slight excess of strong acetic acid (3 or 4 c.c. of 80% acid) and boil for a minute. Cool to room temperature and add 10 c.c. of 30% potassium iodide solution. Titrate at once with thiosulphate solution until the brown tinge has become weak, and then add sufficient starch indicator to produce a marked blue coloration. Continue the titration cautiously until the colour due to free iodine has entirely vanished. The blue colour changes towards the end to a faint lilac. If, at this point, the thiosulphate is added, drop by drop, and a little time is allowed for complete reaction after each addition, no difficulty is experienced in determining the end-point within a single drop. One c.c. of the thiosulphate solution (19 grm. of pure crystals of sodium thiosulphate per litre and standardised against copper foil as above) will be found to correspond to about 0.005 grm. of copper. The weight of dextrose, multiplied by 0.9, gives the weight of the starch.

This method is essentially that of Price, who has reviewed the various methods for estimating starch in meat food products (*U. S. Dept. Agr., Bur. An. Ind., Circular 203*) and has developed a method based on Bigelow's modification of Mayrhofer's method (*U. S. Dept. Agr., Bur. Chem., Bull. 13*, Part 10) and Perrier's method (*Bull. Scien. Pharm., 1908*, 305). The latest details are given in *U. S. Dept. Agr., Bur. Chem., Bull. 162*, p. 97.

(b) The following method is based on the A. O. A. C. method, but has been modified by A. Lowenstein and Richardson (see *Allen's Commercial Organic Analysis*, Vol. VIII, 4th Edition, p. 373).

Fat interferes with the estimation, and if the sample contains much fat, this should first be removed as follows: 10–20 grm. are weighed into a 300 c.c. casserole and dried in the vacuum oven at low temperature for 1.5 to 2 hours. The dried sample is boiled with petroleum spirit and washed until all the fat is extracted and removed. The remaining solvent is then driven off. If the sample is sufficiently lean, the removal of the fat may be dispensed with. In either case the sample is next treated with 50–100 c.c. of an 8% potassium hydroxide solution, covered with a watch glass, and heated on the steam-bath with occasional stirring until the meat is all dissolved. The solution is cooled and 50 c.c. of 95% alcohol are added and thoroughly mixed in. The precipitate is filtered off on asbestos, using a Hirsch funnel (a hump of asbestos in the middle

hastens the filtration), washed twice with a hot solution of 4% potassium hydroxide in 50% alcohol, then several times with 50% alcohol. The precipitate and asbestos are returned to the original casserole and digested with 60 c.c. (more if the starch content is high) of normal potassium hydroxide, which dissolves the starch. The asbestos is filtered off on a Hirsch funnel and well washed with hot water. The filtrate is strongly acidified with acetic acid, made up to 250 c.c., and filtered through a Büchner funnel. An aliquot part of the filtrate is precipitated with an equal volume of 95% alcohol. A Gooch crucible is prepared with a thin layer of asbestos, and on this a layer of prepared sea sand, 2-4 mm. thick, is placed. The crucible is then dried and weighed. The starch is filtered off on the Gooch crucible, washed successively with 50% alcohol, absolute alcohol, and finally ether. The crucible is then dried to constant weight at 100-105°, cooled and weighed.

*Approximate Estimation of Starch.*—A method which can make no claims for accuracy, but which is only roughly approximate, is that of Ambühl (*Pharm. Centralh.*, 1881, 22, 438). It is convenient in combining a qualitative method with an approximate quantitative method. Two grm. of the sausage are macerated with 100 to 200 c.c. water, then boiled for 30 minutes and diluted to 200 c.c. in a volumetric flask. Aliquot portions are pipetted or strained off, cooled, treated with iodine solution in potassium iodide, and the depth of colour compared with standards containing known amounts of the same kind of starch as that in the sample (ascertained microscopically), and boiled for 30 minutes also. The usual allowance must be made when corn-flour or other raw product is present in the product in place of pure starch.

Because of the fact that not only different kinds of starch, but also different kinds of flour, as well as dry bread crumbs, are sometimes mixed with ground meats and sausage, it is difficult for the analyst to estimate the total amount of vegetable matter added in this way, unless a sample of the original material added is obtainable. Since this is seldom possible, the analyst can only report in the majority of cases the percentage of starch actually found.

### **Preservatives in Sausage and Comminuted Meats**

*Boric Acid.*—This is determined as in pickle working on a 100 gram sample for quantities up to 0.2%. Twenty c.c. of 5% barium hydroxide solution are added before evaporating and charring.

*Benzoic Acid.*—Krüger (*Z. Nahr. Genussm.*, 1913, **26**, 12) maintains that most of the previously proposed methods give unsatisfactory results in substances containing a large proportion of protein, difficulty being experienced in extracting the whole of the acid from such foods. He suggests the following method:

Mix 50 grm. of the finely divided meat with 45 c.c. of 70% sulphuric acid and submit to steam distillation. Collect 500 c.c. of the distillate, the flask being heated so as to maintain the volume of the contents as constant as possible. Filter the distillate, make slightly alkaline with sodium hydroxide and evaporate to a small volume. Heat the residual liquid on a water-bath and add potassium permanganate, drop by drop, until the pink colour remains for 5 minutes. Destroy the excess of permanganate with sodium sulphite, evaporate the mixture to 10 c.c., transfer to a separating funnel and acidify with sulphuric acid, rinsing the evaporating dish with sodium sulphite solution and dilute sulphuric acid, and adding the washings to the funnel. Extract the acid solution, which should not exceed 20 c.c., with ether; wash the ethereal extract with water, allow the solvent to evaporate spontaneously in a weighed dish, and weigh the residue after drying for 2 hours over soda-lime. The dried residue may be dissolved in alcohol and titrated with *N*/10 sodium hydroxide. If the weight of benzoic acid is less than 30 mg., Polenske's sublimation method (*Analyst*, 1911, **36**, 584) should be used as a control.

*Sulphurous Acid, Sulphites.*—These are determined by distillation with phosphoric acid in an air-free atmosphere into bromine water, where the sulphurous acid is oxidised to sulphuric and determined as barium sulphate. On account of cystine sulphur and degradation products nearly always present in meat, it is advisable to establish some normal value as a limit for the sulphate obtained from normal meat. A value above this indicates added sulphite.

In Great Britain the amount of preservative permissible in sausages and sausage meat is limited by Schedule I of the Public Health (Preservatives, etc. in Food Regulations) of 1925 to 450 parts of sulphur dioxide per million. Difficulties of determining this amount in foodstuffs have led to systematic studies of the various processes, and the following method has been worked out by G. Monier-Williams. (*Ministry of Health. Reports on Public Health and Medical Subjects.* No. 43. May, 1927.)

**Method of Monier-Williams.**—The method devised by Monier-Williams for the determination of sulphur dioxide in foodstuffs ensures that the whole of the sulphur dioxide is liberated and distilled over without oxidation. It also eliminates errors due to volatile sulphur compounds and organic acids, and economises time, in that a gravimetric determination is necessary only when very small quantities of sulphur dioxide are present.

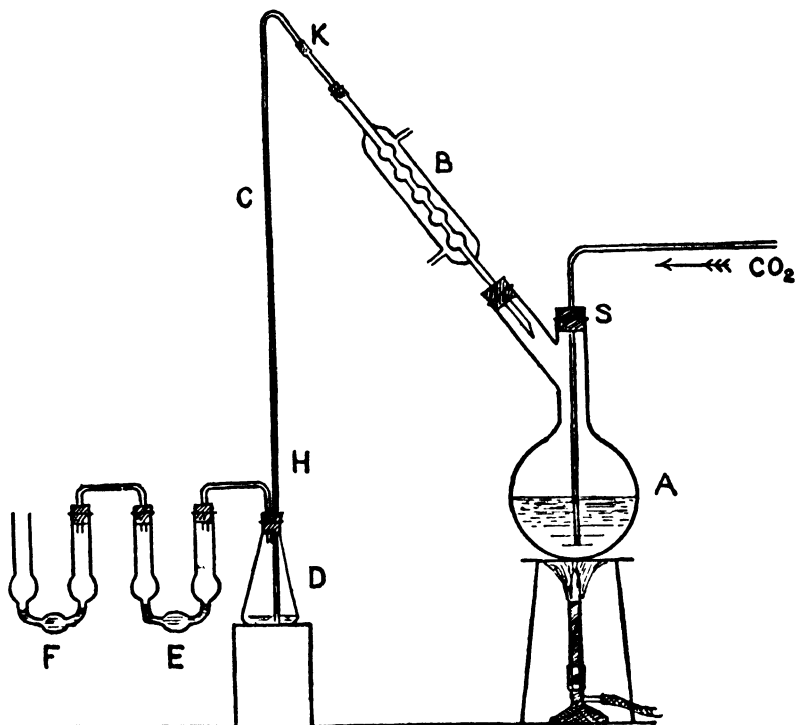


FIG. 24.—Monier-Williams' apparatus for determining sulphur dioxide.

Concentrated hydrochloric acid (20 c.c.) and distilled water (500 c.c.) are boiled in a current of pure carbon dioxide in the 1,500 c.c. flask A, till all air is removed from the apparatus. The flask is then cooled. The sample (usually 100 grms.) is then quickly added, through a tap-funnel in the case of a liquid and the mixture boiled in a slow current of carbon dioxide. In certain cases (*e. g.* cornflour) the flask is first heated in a bath of boiling water in order to avoid cracking it. At the end of 1 hour (*vide infra*) the flow of water in

the reflux condenser (B) is stopped, and any sulphur dioxide is then driven over into the cooled receiver D, which contains 10 c.c. of pure, sulphuric acid free, 10-volume (3%) hydrogen peroxide.

The Peligot tube, E, contains a similar solution, and the tube F, which contains 5 c.c. of a mixture of hydrogen peroxide and barium chloride solutions acidified with hydrochloric acid, acts as a guard-tube for any excess of sulphur dioxide, but should however not usually be necessary.

Rubber stoppers are used throughout. As soon as the tube at the point H is hot to the touch, the receivers are disconnected at K, the contents all washed into D, and the sulphuric acid produced titrated at room temperature with 0.1 *N* sodium hydroxide solution. Brom-phenol blue is preferable to methyl orange as indicator, since it gives a sharp colour change and is unaffected by carbon dioxide and traces of volatile organic acids. If a gravimetric determination is required as a check, the barium sulphate must be precipitated and filtered in the cold, or the presence of volatile sulphur compounds may lead to fictitiously high results. The precipitate should be washed by decantation with hot water before filtration.

The method has been tested on solutions containing known amounts of sulphur dioxide, and the titration and gravimetric methods have been shown to be in satisfactory agreement. There was evidence, however, of oxidation during the distillation, as slightly low results were obtained in some cases. Acetaldehyde in amounts in which it may be normally present (*e. g.* in wines) does not affect the results, and hydrochloric, benzoic, salicylic, acetic and cinnamic acids do not pass over into the distillate from the flask. Pre-treatment with alkali (sodium bicarbonate or hydroxide in the presence of carbon dioxide) has been previously advocated, but does not appreciably affect the results when hydrochloric acid (30 c.c.) is used instead of phosphoric acid.

Since 1 c.c. of 0.1 *N* sodium hydroxide solution corresponds with 32 parts of sulphur dioxide per million on 100 grms. of sample, the gravimetric method is recommended when the titration figure is less than 0.5 c.c.

*Method of the Chemists' Committee of the Manufacturing Confectioners' Alliance and of the Food Manufacturers' Federation.*—A committee of chemists engaged in the food manufacturing interests in Great Britain investigated the methods of determining sulphur

dioxide, with the object of selecting accurate methods which should be sufficiently rapid for works' control purposes (*Analyst*, 1928, **53**, 118).

It was found that low results, commonly obtained, were due to two principal causes, viz. (a) oxidation of sulphur dioxide in the distilling flask; (b) escape of sulphur dioxide with air in the receiving vessel. The rapidity with which both alkaline and acid sulphite compounds oxidise when their solutions are exposed to the air, particularly at the higher temperatures, is not sufficiently realised. For example, soaking gelatin for even 15 minutes preparatory to distillation results in a decided loss of sulphur dioxide. The essential point about the ordinary method of determining sulphur dioxide by distillation is that, if the evolution of the gas from the liquid is slow, loss occurs by oxidation. If it is rapid, loss occurs through the air containing the sulphur dioxide bubbling too rapidly through the oxidising solution.

The process recommended by the Committee eliminates the second source of error, whilst the first is eliminated by distilling as rapidly as possible.

When sulphides are present, the Committee suggests that the method of Monier-Williams (see p. 442) should be used, but that for normal works' laboratory purposes more rapid methods are desirable.

*Normal Procedure and Apparatus, Preparation of the Sample.*—In all cases samples should be divided as finely as is compatible with prevention of loss of sulphur dioxide. Flake gelatin should, if possible, be ground to a fine powder. The variations in the sulphur dioxide content in different parts of a flake gelatin are considerable. There should be no preliminary soaking in water, as this is known to give rise to oxidation.

Sausage meat should be well broken up by stirring in the flask with a long glass rod.

The loss of sulphur dioxide with the lapse of time is considerable with many articles, and this is frequently the explanation of disagreement between two analysts.

*The Flask.*—The flask should normally be of 500 c.c. capacity. It should be of resistance glass, as it is to be heated with a bare flame. It should be round in shape, as there is a danger of the froth being carried over into the distillate if a conical flask be employed.

The rubber bung must fit tightly, and should not be new. It holds a tap funnel, the still-head, and, where necessary, the inlet-tube for steam.

*The Still-head.*—The Reichert-Meissl and Kjeldahl still-heads have both been found unreliable when the quantity of steam passing is great. A new type has been designed which is better adapted to the process, and is to be obtained from Scientific Supplies Co. Ltd., 52 Hatton Garden, London, E.C.1. It is sold as the "B.A.R. Still-Head," and consists of two bulbs, each approximately  $1\frac{1}{2}$ " wide, the diameter at the junction being about  $\frac{7}{8}$ ". The tube below the lower bulb is about  $\frac{1}{2}$ " in diameter and 4" long, the end being cut off obliquely, as in filter funnels. There is a hole of from  $\frac{1}{8}$ "– $\frac{1}{4}$ " diameter, one inch from the tip.

The tube above the bulbs is of  $\frac{1}{4}$ " diameter, and is bent over almost at right angles just above the upper bulb. The horizontal portion is about 6" long. It is then bent over at right angles again, the remaining portion being  $2\frac{1}{2}$ " long. This end is fitted into the condenser.

*The Condenser.*—The condenser is fixed vertically. The jacket should not be less than 18" long, and the condenser tube  $\frac{1}{2}$ " in diameter. If condensation is not sufficiently good, a longer condenser must be used. The temperature of the condensate should be as low as possible and certainly should not exceed  $27^{\circ}$  C. The adapter is fitted to the end of the condenser tube.

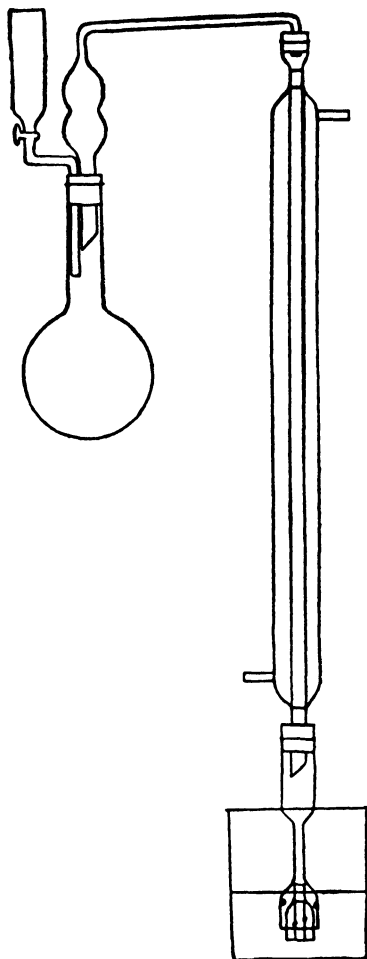


FIG. 25.—Chemists' Committee's apparatus for determining sulphur dioxide.



*The Adapter.*—The adapter is of a bubbler or “scrubber” type, and is sold by Scientific Supplies Ltd., as the “B.A.R.” adapter. It consists of an upper cylindrical portion, into the top of which the end of the condenser fits, about 1" in diameter and 3" long. To the bottom of this is fitted a tube  $\frac{1}{4}$ " in diameter and 4" long. At the end of this is an extension surrounded by a bell-shaped portion about  $1\frac{1}{2}$ " long and 1" wide. The bottom of this is level with the end of the tube. In it are two holes opposite each other, about  $\frac{1}{8}$ " in diameter, and placed  $\frac{3}{4}$ " from the bottom. Outside this is a further bell-shaped portion about  $1\frac{3}{8}$ " in diameter. The bottom of this is about  $\frac{3}{8}$ " above the bottom of the inner bell. Seven-eighths of an inch above it are two holes opposite to each other. A line through these two holes is at right angles to a line drawn through the two holes in the inner bell.

It has been proved that this device is an efficient scrubber for the rapidly emerging gas.

*The Burner.*—The burner should be of a powerful type, as it is necessary to heat the contents of the flask to boiling point within  $2\frac{1}{2}$  minutes. An asbestos screen is arranged to protect the condenser from the heat of the burner.

*Water Used in the Flask.*—The water used should be distilled and must be de-aerated by boiling for not less than 15 minutes. After boiling it should be both cooled and stored in an atmosphere of carbon dioxide. There is good reason to believe that appreciable losses are due to the oxidation of sulphur dioxide by dissolved oxygen.

*Acid.*—Although phosphoric acid is conveniently employed with most substances, it has been found that for starch and gelatin hydrochloric acid is necessary. With hydrochloric acid, however, there is more danger of charring. It has been found to give more rapid evolution of sulphur dioxide from gelatin, and may prove preferable to phosphoric acid in other cases.

*Oxidation of the Distillate.*—Iodine was selected as the oxidising substance, because it was desired that the process should normally be volumetric, although capable of being followed by a gravimetric determination, if desired. Hydrogen peroxide was not adopted because of its known action on sulphides when hot. Monier-Williams has now shown that, in the cold, hydrogen peroxide does not oxidise appreciable amounts of hydrogen sulphide or volatile organic matter to sulphuric acid. This fact was, of course, unknown to the Committee at the time.

Monier-Williams has also rendered the hydrogen peroxide method capable of volumetric adaptation by means of the use of a reflux condenser, which prevents volatile organic acids distilling over and renders possible the titration of the sulphuric acid formed in the receiving vessel.

The use of the reflux condenser naturally renders the process of distillation somewhat slow, and the Committee therefore still considers that for works' laboratory purposes the use of iodine has much to recommend it.

*The Receiving Vessel.*—The receiving vessel is a 600 c.c. beaker. The bottom of the adapter should be not less than  $\frac{3}{8}$ " from the bottom of the beaker, in order to prevent bubbles of gas passing outside the bells.

*Heating and Distillation.*—The Committee desires to emphasise the fact that there is a great liability for the sulphur dioxide to be oxidised during the heating of the liquid. It is essential that the liquid should boil within  $2\frac{1}{2}$  minutes.

Distillation must also be rapid. It is found with most substances that the sulphur dioxide comes over within a few minutes. If it is found that more than ten minutes distillation are required, as is frequently the case with dried fruits for example, steam distillation should be employed.

With most food materials there is, on distillation with acid, a continuous, though small, evolution of iodine-decolorising substances, even when no sulphur dioxide is present. It is, therefore, necessary to limit the time of distillation, apart from the desirability of distilling quickly in order to avoid oxidation. The end-point of distillation may usually be taken as that at which more than one minute is required to decolorise 0.1 c.c. of *N/20* iodine.

*Volumetric and Gravimetric Determinations.*—It is anticipated that even where reliance is placed chiefly on the gravimetric process, a volumetric determination will usually be made as a check.

*Method of Determination.*—Sufficient distilled water is placed in the receiving beaker to cover the outer bell of the adapter. To this is added 0.2 to 0.3 c.c. of a filtered 1% starch solution, and a few drops of *N/20* iodine. Where a high percentage of sulphur dioxide is anticipated a larger quantity, *e. g.* 1 to 5 c.c. of *N/20* iodine may be added.

Two hundred c.c. of de-aerated water are placed in the distilling flask, followed by 25 to 100 grms. of the sample. The bung holding the still-head etc. is inserted as rapidly as possible, and it is of course essential that it should fit well.

Twenty five c.c. of 20% phosphoric acid are then run in through the funnel, and the contents of the flask heated to boiling as rapidly as possible by means of the naked flame of a powerful burner. The time taken to effect boiling should not exceed  $2\frac{1}{2}$  minutes.

*Volumetric Process.*—As the distillation proceeds, *N*/20 iodine is added from a burette fixed over the receiving vessel, so that the colour remains. It is, in most cases, undesirable to add a considerable excess of iodine during the volumetric process, as there is a danger of loss of iodine. The distillate should be cool, and its temperature should not exceed  $27^{\circ}$  C.

Generally, at least 90% of the sulphur dioxide comes over in the first rush of gas, and only traces after five minutes' boiling. The end-point of the distillation was at first taken as that at which the colour due to 0.1 c.c. of *N*/20 iodine persists for more than 2 minutes, but it is probable that one minute would be a better time limit for this amount, with, possibly, some exceptions. The distillation should be complete within 10 minutes' boiling. Too prolonged boiling may produce reducing substances other than sulphur dioxide. If it is found that this time is exceeded, even when the most powerful flame practicable is used, recourse should be had to steam distillation.

*Gravimetric Process.*—The distillate is filtered and brought to a volume of 200 to 250 c.c. If it is necessary to boil down to this volume, concentration should be carried out on an electrically heated plate, or precautions should be taken to prevent sulphur dioxide from the gas flame being absorbed. Ten c.c. of *N*/10 hydrochloric acid are then added, and the liquid is boiled for five minutes; 2.5 c.c. of 10% barium chloride solution are next added, drop by drop, and the solution boiled for five more minutes. Two and a half c.c. more barium chloride solution are added and the liquid is allowed to simmer for one hour, finally being allowed to cool. It should then stand for at least two hours, or preferably over-night, and finally be filtered either through a No. 40 Whatman filter paper or by means of paper pulp. After washing and drying, the paper and precipitate are ashed as usual.

A blank determination should be made with the chemicals if special accuracy is desired.

H. Osman Jones has devised the following method for the *determination of Sulphur Dioxide in Sausages* (*Analyst*, 1928, **53**, 138).

Sodium metabisulphite is added to a 35-lb. chopping of sausage meat in an amount equivalent to 450 parts of sulphur dioxide per million.

It should be noted that there is always an almost immediate loss of sulphur dioxide in a sausage when the sulphite is added to it; when 450 parts per million are added the loss is very approximately 150 parts, so that the amount of sulphur dioxide found is invariably lower than that added. Further, when working on a manufacturing scale it is a difficult matter to obtain an absolutely uniform mixture; some discrepancy in two or more determinations on the same sample is therefore to be expected.

*Method.*—Twenty c.c. of  $N/20$  iodine solution are diluted to about 80 c.c. and introduced into the receiving flask, the delivery tube from the condenser being so arranged that it will dip beneath the surface of the iodine solution. An appropriate quantity of the substance to be examined (usually 50 grms.) is placed in the distilling flask, together with about 250 c.c. of freshly boiled and cooled distilled water and 20 c.c. of a 10% solution of phosphoric acid. The apparatus is connected together and carbon dioxide passed through it for ten minutes, after which time the supply is reduced and the vacuum formed. In order to prevent bumping and foaming, it is desirable to maintain as rapid a stream of carbon dioxide as possible consistent with a 20-inch vacuum. As soon as the evacuation is started a bath of hot water is introduced under the distilling flask.

About 100 c.c. are distilled, and the vacuum reduced by means of the screw clip on the release. Care is necessary here to prevent sucking back of the distillate into the apparatus. When the pressure is normal the source of heat is removed, the condenser washed through with a little water, and the liquid in the receiving flask (to which has been added the potassium iodide in the trap) titrated with  $N/20$  sodium thiosulphate in the usual manner.

The following are some results obtained by six members of the Committee. The figures are given as a percentage of the amount of sulphur found in a sample of metabisulphite by direct titration. (Amount found by direct titration equals 100%.)

	Gravimetric %	Volumetric %		Gravimetric %	Volumetric %
A.....	99.5	96.2	D.....	97.8	96.1
B.....	100.7	99.5	E.....	101.2	97.7
C.....	94.7	94.6	F.....	99.7	99.7

It should be noted that no iodine trap was used when the above results were obtained.

*Comparative Results.*—During the time that had elapsed since the method was tried by the Committee, Osman Jonas had opportunity of applying it to the routine samples of sausages, and he found that, with practice, the process is not so difficult of manipulation as was thought, and that it gives good results.

The following figures show the results of determinations on samples by various methods, all determinations being made volumetrically.

	Sulphur dioxide %		Sulphur dioxide, parts per million
Sodium metabisulphite		Sausages	
Vacuum method . . . . .	62.08	Leach's method . . . . .	179.2
Committee's method . . . . .	61.90	Committee's method . . . . .	192.0
Monier-William's method . . . . .	60.50	Vacuum method . . . . .	187.5
Direct titration . . . . .	63.04	Sausages	
	Parts per million	Leach's method . . . . .	153.6
Gelatin		Committee's method . . . . .	179.2
Vacuum method . . . . .	1011	Vacuum method . . . . .	179.2
Committee's method . . . . .	1011	Monier-William's method . . . . .	172.8

A modification of this method would be to substitute hydrogen peroxide for standard iodine, and then determine the amount of sulphur dioxide either gravimetrically or volumetrically.

For other methods of determining preservatives see the *Methods of Analysis of the A. O. A. C.*, 1925, p. 125.

### Horse Flesh Sausages

On the continent of Europe, and especially in France, horse flesh is extensively used for the manufacture of sausages, the vendors of which are required to indicate the nature of the articles they sell. Hence a means of recognising horse flesh, and detecting it when mixed with the flesh of other animals, is of practical importance.

The physical characters of horse flesh have been employed for its recognition (see p. 243), but are not very conclusive under any circumstances, and are useless when the flesh is minced and mixed with other kinds of meat, as in sausages.

In addition to the precipitin method (page 244), the determination of glycogen has been used to identify sausages containing appreciable amounts of horse flesh. This fact has been utilised by Brautigam and Edelmann (*Chem. Centralb.*, 1894, **1**, 485; *Analyst*, 1894, **19**, 24) for its detection in sausages. They state that 10% of horse flesh or horse-liver can be detected by this means, the proportion of glycogen therein ranging from 0.37 to 1.07%, whilst the flesh of other animals used for food contains little or none—ox flesh coming next with 0.20%. On the other hand, the flesh of the foetus, both of man and of the lower animals, is rich in glycogen. M. Humbert (*J. Pharm. Chim.*, 1895, 195; abst. *Analyst*, 1895, **20**, 95) confirms the value of the observation of Brautigam and Edelmann and recommends the following method of procedure: About 50 grm. weight of the muscular tissue should be cut into small pieces and boiled for an hour with 200 c.c. of water. After cooling, nitric acid is added in the proportion of 5 c.c. to 100 c.c. of broth, and the liquid filtered. To a portion of the filtrate contained in a test tube, iodine water is added, drop by drop, so as not to mix the liquids, when the formation of a reddish-violet zone at the junction of the two strata will indicate the presence of glycogen in the original sample. The reagent should be recently prepared by saturating hot water with iodine. In the original form of the test, Brautigam and Edelmann employed hydriodic acid instead of the hot iodine water recommended by Humbert. The reaction is said to be quite characteristic of horse flesh. Humbert states that of 10 samples of horse flesh obtained from different dealers in Paris, seven showed the colour very plainly, in two it was less pronounced but distinct, while in one case it was doubtful. In no instance was there any coloration with beef, veal, mutton, or pork. Beef-broth left in contact with the iodine for 10 days gave no reaction. The flesh of the ass gave a negative result, but with that of the mule the reaction was the same as with horse flesh. A mixture of equal parts of horse flesh, beef, mutton, veal, and pork showed the coloration, but it was less pronounced than with horse flesh alone. (Compare pp. 258, 264.)

Courlay and Coremons (abst. *Analyst*, 1896, **21**, 231) recommend a slight modification of the foregoing test. About 50 grm. of the substance, in as fresh a state as possible, should be finely divided and boiled for 15 to 30 minutes with 20 c.c. of water. After cooling (*no* addition of nitric acid being made) the broth is filtered and then

tested with a few drops of iodine solution, prepared by dissolving 2 parts of iodine and 4 of potassium iodide in 100 parts of water. A brown coloration, disappearing on warming to  $80^{\circ}$  and reappearing on cooling, shows the presence of horse flesh. Courlay and Coremons state that, by their modification of the iodine test, no glycogen reaction was obtained from the flesh of calves, pigs, dogs, or cats; but that this observation does not apply to the foetus of any of these animals. In the presence of starch (*e. g.*, in sausages) the blue reaction with iodine may entirely mask the brown coloration due to glycogen; but this is obviated by treating the broth with two or three times its measure of strong acetic acid, filtering, and applying the iodine test to the filtrate.

W. Niebel (*Chem. Centralb.*, 1893, page 323; abstr. *Analyst*, 1895, 20, 252) criticises the foregoing methods on the ground that the reaction with iodine is uncertain, since glycogen is also found in the flesh of dogs, cats, and very young calves, in the livers of cattle, and in meat extract to the amount of 1.5%. With old sausages from horse flesh Brautigam and Edelmann always obtained the glycogen reaction, although that substance would usually be completely decomposed under these circumstances. There is also an uncertainty in the reaction caused by the fact that the erythro-dextrin formed from the starch gives a similar coloration with iodine, and no means of removing it is known. Niebel considers that the red colour with iodine is not sufficient proof of the presence of glycogen, which should be isolated in a pure condition. Nevertheless, the iodine coloration, and the occurrence of more than 1% of glucose in the fat-free substance, points to the presence of horse flesh in a sample, even when all the glycogen has been decomposed. The red colour only fails in the case of the flesh of young foals.

The figures given on p. 259 were obtained by Bujard more recently. Some of the samples were analysed both by the method of Niebel and Salkowski and by that of Mayrhofer (*Forsch. Ber.*, 1896, 141), to which he gives the preference on the ground of simplicity. In the latter process, the finely divided flesh is dissolved in aqueous potassium hydroxide, the proteins precipitated by hydrochloric acid and Nessler's solution, and the clear filtrate treated with alcohol. This throws down the glycogen, which is filtered off, washed with dilute alcohol and ether, and dried at  $110^{\circ}$ .

In Bujard's opinion, his figures show that only in exceptional cases (where the amount is large) can the glycogen be taken as conclusive of the presence of horse flesh, especially when the latter is mixed with other kinds of flesh.

The methods of Pflüger have now, to a large extent, superseded the older methods for the estimation of glycogen. These are given in detail on pages 259 and 264.

James Bell has pointed out (*Chem. News*, 55, 15) that the fat of the horse differs materially in its characters from that of the ox. Thus the fat isolated from different parts of the horse—such as the round, flank, ribs, kidneys, and heart—was found to have a sp. gr. at 38° ranging from 0.9086 to 0.9088. The intramuscular fat had a sp. gr. of 0.9084 at the same temperature, and hence was not greatly different in character from that obtained directly from the adipose tissue. The horse fat melted to a clear oil at 70°, and the amount of solid fat deposited at a lower temperature was comparatively small. A series of similar experiments made with beef fat showed a m. p. of 110 to 116° F. The sp. gr. was taken at 120° F., and when the results were corrected to 100°, to render them comparable with those obtained with horse fat, the sp. gr. was found to range from 0.9036 to 0.9040. These figures show a substantial difference between beef fat and horse fat, and the distinction is still more marked in the case of mutton-fat. The low melting-point of horse fat is an important characteristic, and in cases where the flesh is not of mixed origin ought usually to be conclusive as to its nature.

R. Frühling (*Z. ang. Chem.*, 1896, 352; abst. *Analyst*, 1896, 21, 231) was led to study the fat of horse-flesh, being unable to obtain conclusive results by the reaction for glycogen. He found the fat extracted from sausages made wholly from horse flesh to have an iodine absorption of 72.5%, while that from sausage composed of horse flesh with 15% of pork gave 62.3; and from sausage composed of equal parts of horse flesh and pork, 57.2%. As pure pork-fat (lard) shows an iodine absorption averaging about 60, it is evident that the method is inconclusive.

H. Bremer (*Forsch. Ber.*, 1897, 4, 1; abst. *Analyst*, 1897, 22, 104) has reviewed the work of other chemists, and proposed a method of detecting horse flesh, based on the characters of the intramuscular fat.

The subjoined table gives the results obtained by his method:



	Iodine value of intra- muscular fat	Iodine value of liquid fatty acids
I. Horse-flesh sausage without bacon.....	75.8	108.1
II. Horse-flesh sausage with about 6% of bacon .....	74.0	104.1
III. Horse-flesh cervelat (brain) sausage with about 22% of well-smoked bacon.....	53.7	92.4
IV. Horse-flesh cervelat sausage with about 25% of bacon.....	74.1	102.1
V. Ordinary sausage with some bacon.....	57.6	94.2
VI. Thuringian cervelat sausage with about 65% of pig's fat.....	64.3	95.8
VII. Mixture of I and V in equal parts.....	66.4	103.1
VIII. Mixture of IV and VI in equal parts.....	65.2	99.5

It is stated that whenever horse-flesh is present the petroleum spirit extract has a red to reddish-brown colour, and that even the liquid fatty acids have a more or less reddish-yellow shade. On the other hand, bull's flesh gives a similar colour, so that too much reliance must not be placed on this fact, except as a confirmatory test. When, however, this is found to be the case, when at the same time glycogen is detected, and when the iodine value of the intramuscular fat exceeds 65, and that of the liquid fatty acid is considerably over 95, there can be but little doubt as to the presence of horse flesh.

For other methods bearing on the identification of species see pages 243 and 523.

### Artificial Colour in Sausage

In the United States the use of artificial colour in meats and meat products is forbidden by the regulations of the Bureau of Animal Industry. The casings of sausage or other meats may be coloured by dipping or cooking as shown above (p. 392). Of the various permitted colours, the following are used by some branches of the meat-packing industry:

56 Ponceau 3 R  
107 Amarant  
517 Erythrosine

85 Orange I  
94 Tartrazine  
692 Indigo disulpho acid

Of the total amount used, probably 90% is Amaranth and Orange I, with Tartrazine as third choice. Artificial dyes are now less used than formerly to colour sausage meat.

**Methods of Detecting Artificial Colour in Sausage.**—The methods of identifying colours when used in food products, and especially in meat, are imperfect, even when it is certain that an added dye is present. The subject is complicated by the difficulties of extraction and by the colour found in meat which has been cured with saltpetre. Ground meat when exposed to the air rapidly turns to a dirty drab, especially in the presence of salt, and the artificial colour was formerly added to maintain a red colour. Saltpetre accomplishes the same result (page 403).

*Red ochre* is said to have been used, but would make its presence evident by excessive iron in the ash of the material.

For the detection of *cochineal-carmine* in sausages, Klinger and Bujard (*Z. angew. Chem.*, 1891, 4, 515; abst. *J. Chem. Soc.*, 1893, 63, 56) recommend that about 20 grm. of the cut-up sausage should be heated in a water-bath with a mixture of equal parts of water and glycerin. In the absence of this colouring matter only a slight yellow colour is produced, but in its presence the liquid becomes decidedly reddish in colour. The filtered solution is heated, if necessary, with another 20 grm. of the sausage. The clear liquid is then examined in the spectroscope, when cochineal-carmine can be readily recognised by its characteristic absorption-bands lying between *b* and *D*. A preferable plan is to precipitate the colouring matter as a lake, and, after washing, to dissolve it in a little tartaric acid. A more concentrated solution is thus obtained, and the spectroscopic test is consequently more satisfactory.

H. Bremer (abst. *Analyst*, 1897, 22, 216) confirms the value of the foregoing method for the detection of cochineal-carmine. He heats the finely divided sausage for several hours on the water-bath with two volumes of the slightly acidified glycerin-water mixture. The yellow solution is freed from fat and filtered, and the colouring matter precipitated as a lake by the addition of alum and ammonia. The absorption-bands lying between *b* and *D*, which are characteristic of carmine-lake, may be readily observed in the spectroscope. In one instance Bremer found a cervelat sausage coloured with cochineal-carmine to have all the appearance of good meat when cut, but further examination showed it to be quite unfit for food, the acid

value of the fat being 76.0 (*i. e.*, the separated fat required 7.6% of potassium hydroxide for its neutralisation). *Aniline red* has been detected, but certain varieties of *Benzopurpurin* (see Vol. V) are now most commonly employed. This colouring matter is allied to congo red, and has the property of dyeing vegetable fibres without a mordant. It is added to the meat together with the dry bread or biscuit, which it effectually colours.

*Aniline red*, if present, can be detected by extracting the finely divided sausage with methyl alcohol, evaporating the solution after straining or filtering, taking up the residue with water, and immersing white wool in the boiling liquid, when the fibre will be dyed red if Rosaniline is present (See *Circulars* 25, 35, and 63, *Bur. Chem. U. S. Dept. Agric.; Bull.* 107 Rev. *Ibid.*, page 190; and *Allen*, Vol. V, p. 623 *et seq.*).

Weller and Riegel (*Forsch. Ber.*, 1897, 4, 204; abstr. *Analyst*, 1897, 22, 324) state that the haemoglobin of pig's blood is converted by nitre into a modified form which dissolves with red colour in diluted glycerin, alcohol, amyl alcohol, and ether, but can be distinguished from cochineal-carmine by its absorption spectrum after reduction by ammonium sulphide. (See Vol. V.)

In attempting to extract vegetable and coal-tar dyes from meat, it is often necessary to try several solvents before a satisfactory one is found. Besides those already enumerated, acidified glycerin or alcohol, a 5% solution of salicylic acid, and dilute ammonia will be found useful. After filtering off the solvent, it is concentrated to small bulk, acidified with hydrochloric acid, and white, grease-free wool dyed in it. If the wool is dyed distinctly red, a coal-tar dye is probably present, and further examination should be made.

Very dilute extracts may be concentrated by precipitation of the dye as a lake, dissolving in hydrochloric acid and making alkaline with ammonia water.

*Methods of the A. O. A. C.*—Amaranth, Orange I, and Tartrazine are all soluble in water, and so the procedure for water-soluble coal tar dyes is applicable. Sausage casings may contain these dyes.

Macerate 20–200 grm. of the sample with 4 to 5 times its weight of alcohol (80% by volume). After standing a few hours, pour off the solvent as completely as possible and repeat the extraction, using alcohol of 70% by volume and containing approximately 1% of ammonium hydroxide. Examine separately the filtered extracts

by boiling or heating on a steam bath with a small piece of white woollen cloth (nun's veiling). Or boil the ammoniacal solution until practically neutral, complete the neutralisation with acetic acid, add the neutral 80% alcohol extract, continue the evaporation until most of the alcohol is removed, and boil a small portion with wool.

*Methods of Sostegni and Carpentieri* (*Z. anal. Chem.*, 1896, **35**, 397).—The method of Sostegni and Carpentieri and Arata's method for coal-tar colours are not reliable in the presence of archil, archil derivatives, and sulphonated indigo, as these substances give dyeing reactions not to be distinguished from coal-tar colours. It is, however, comparatively easy to detect archil. A red colour turning purple with dilute ammonium hydroxide, reduced by zinc and hydrochloric acid and easily reoxidising in the air, is either archil or a closely related colour (*Tolman, J. Amer. Chem. Soc.*, 1905, **24**, 25). Archil (orchil) can be extracted from an ammoniacal solution by amyl alcohol, but the sulphonated archil colours now on the market do not respond to the test. Indigo is used in many green and violet colours and can be recognised as described in Vol. V, 425, *et seq.*

Dissolve or extract from 10 to 20 grm. of the sample in 100 c.c. of water, filter if necessary, acidify with from 2 to 4 c.c. of a 10% solution of hydrochloric acid. In this solution immerse a piece of woollen cloth, which has been washed in a very dilute solution of boiling potassium hydroxide and then washed in water, and boil for 5 to 10 minutes. Remove the cloth, thoroughly wash it in water, and boil in a very dilute hydrochloric acid solution. After washing out the acid dissolve the colour in a solution of ammonium hydroxide (1:50). With some of the dyes solution takes place quite readily, whilst with others it is necessary to boil for some time. Take the wool out, add a slight excess of hydrochloric acid to the solution, immerse another piece of wool, and boil it again.

With vegetable colouring matter this second dyeing gives practically no colour, and there is no danger of mistaking a fruit colour for one of coal-tar origin. It is absolutely necessary that the second dyeing should be made, as some of the coal-tar dyes (*U. S. Dept. Agr., Bureau of Chemistry, Bull.* **66**, p. 24) will dye a dirty orange in the first acid bath which might be easily passed for vegetable colour, but after solution in alkaline bath the second acid bath dyes a bright pink.

*Arata's Method* (*Z. anal. Chem.*, 1889, **28**, 639).—This method gives results comparable with those of the first dyeing of the preceding method. It was recommended for detecting coal-tar colours in wine, and has been used by Winton (*Conn. Agr. Exper. Stat. Rept.*, 1899, Part 2, p. 131) for fruit products.

Boil from 20 to 30 grm. of the sample dissolved in 100 c.c. of water for 10 minutes with 10 c.c. of a 10% solution of potassium hydrogen sulphate and a piece of white wool or woollen cloth, which has been previously heated to boiling in a very dilute solution of sodium hydroxide and thoroughly washed in water. After removal from the solution, wash the wool in boiling water and dry it between filter papers.

In addition to this, it is advisable in all cases to dissolve out the colouring matter with ammonium hydroxide, as in the first method, and dye again after acidifying with a few drops of hydrochloric acid, since Arata's method gives practically the same results as the first dyeing in hydrochloric-acid bath and needs to be confirmed by the second dyeing.

Another advantage in the second dyeing is that if a large piece of woollen cloth is used in the first dyeing, and a small piece in the second dyeing, small amounts of colouring matter can be brought out much more decidedly in the second dyeing, where practically all of the vegetable colouring matter has been excluded. The colouring matter can be identified to a certain extent by the schemes of Witt (*Z. anal. Chem.*, 1887, **26**, 100), Allen, Weingärtner (*Z. anal. Chem.*, 1888, **24**, 232-249), Dommergue (*Z. anal. Chem.*, 1890, **29**, 369-377), Girard and Dupré (*Analyse des Matières Alimentaires*, etc., 583-593), and Rota (*Analyst*, 1899, **24**, 41). The tests can be made directly on the dyed fabric, or the dye can be dissolved out (*Z. anal. Chem.*, 1889, **28**, 639; Borgmann, *Analyse des Weines*, p. 91; Winton, *Conn. Agr. Exper. Stat. Rept.*, 1899, Part 2, p. 131). To remove the colour, wash the wool with dilute tartaric acid and then with water and dry between filter paper. Saturate the wool with strong sulphuric acid, press out the colour with a glass rod after 5 to 10 minutes, and dilute to 10 c.c. with water.

Remove the wool, make the solution alkaline with ammonium hydroxide, and when cold extract with 5 to 10 c.c. of amyl alcohol. Separate the amyl alcohol, evaporate it to dryness, and test the residue with strong sulphuric acid.

*Ponceau R*, 2R, 3R, S and 3S give yellow red to carmine red.

*Ponceau S* and *Tropæolin O* give yellow to orange-yellow.

*Biebrich scarlet* gives a green; *Bordeaux red* and *Crocein Scarlet* give blue; *Tropæolin OOO* and *Solid Red* give violet.

If the wool is well dyed, most of these colours may be obtained on the fabric.

These are the reactions of only a few of the more common colours; in order to carry the work further the more complete works mentioned must be used.

*Methods for the Separation and Estimation of the Permitted Coal-tar Colors.* Price (*Circular 180, Bur. Animal Industry, U. S. Dept. Agric.*) proposed a method for the separation of the seven colours: *Amaranth*, *Ponceau 3R*, *Erythrosin*, *Orange I*, *Naphthol Yellow S*, *Light Green S F Yellowish*, and *Indigo Disulpho Acid*.

There are now 14 permitted colours. The methods for separation and estimation have been set forth in detail by Mathewson (see Vol. V, pp. 425-508) and in the *Methods of Analysis of the A. O. A. C.* (*Methods of Analysis* 1925, pp. 139-169). In both places bibliographies will be found. It seems unnecessary to repeat the details here.

The chemistry and analysis of the dyes themselves is treated at length by Ambler, Clarke, Evenson, and Wales (*U. S. Dept. Agr., Bull. 1390*, 1926) and by Evenson and Herrick (*U. S. Dept. Agr., Bull. 1390*, Suppl. 1, 1930).

**British Public Health (Preservatives, Etc., in Food) Regulations.** *Regulations.*—By the British Ministry of Health Regulations of 1925 it is permissible to use any pure synthetic dyestuff, with the exception of five, which are specifically excluded. These are *Picric Acid* (7), *Victoria Yellow* (8), *Martius yellow* (9), *Aurantia* (12), and *Aurine* (724).<sup>1</sup>

Nicholls (*Analyst*, 1927, 52, 585) has published a scheme for the detection of these prohibited colours in foods:

An ammoniacal extract of the foodstuff is neutralised to methyl orange with acid, and then acidified to the extent of  $N/100$  to  $N/50$ . It is next extracted twice with methylated ether, the extracts being transferred to a separating funnel. The ether is extracted with successive quantities of about 5 to 10 ml. of approximately  $N/100$  sodium hydroxide solution until no more colour is removed. An

<sup>1</sup> The number in brackets refer to the corresponding number in the Colour Index of the Society of Dyers and Colourists, 1924.

equal quantity of petroleum spirit is added to the methylated ether, which is again extracted with dilute sodium hydroxide solution.

Absence of colour in the alkaline layers indicates absence of prohibited dyes:

Extract from methylated ether is coloured yellow by picric acid: yellow to orange by Victoria yellow, Manchester yellow and gamboge; red by aurine.

<p>Extract from petroleum spirit and methylated ether is orange-red if aurantia is present. Apply tests 10 and 19.</p>	(A) To 1 ml. of solution add 1 ml. alcohol, 1 ml. meth. ether and 1 ml. 30% sodium hydroxide solution in that order, and shake.	
	Ethereal solution is colourless and soda solution is pink if aurine is present. Confirm by tests 11 and 22.	Ethereal solution is yellow and soda solution is colourless if others are present.
	(B) To 1 ml. of solution add 2 ml. of 30% sodium hydroxide solution, mix and leave for 1 minute. Add 1 ml. methylated ether and 1 ml. alcohol and shake.	
	Ethereal solution is colourless and soda solution is (a) yellow if picric acid is present, (b) pink if aurine is present. A yellow colour in the soda layer may not be seen if both are present. In that event apply the confirmatory tests for picric acid, viz., tests 3, 9, 11, and the formation of picrates with naphthalene, quinine, etc.	Ethereal solution is yellow and soda solution colourless if others are present.
	(C) To original solution add acid and extract with petroleum spirit.	
	<p>Ether coloured yellow indicates gamboge. Confirm by tests 21 and 13, 14, 15 (if necessary concentrating the solution).</p>	<p>Victoria yellow and Manchester yellow give colourless petroleum spirit, but on removing this and adding petroleum spirit which has been shaken with ammonia a yellow turbidity is obtained. (Gamboge also gives this.)</p>
		<p>Victoria yellow. Apply test 12.</p> <p>Manchester yellow. Apply tests 16 and 17.</p>

Jamieson and Keyworth (*Analyst*, 1928, **53**, 418) have shown that, when treated with certain reagents, these dyestuffs give sparingly soluble crystalline precipitates, which enable them to be recognised in the minute proportions in which they are commonly used in food (about 1 part in 2,000).

The dye is first extracted by the methods given in Vol. V. of this work (pp. 431-434).

If necessary, dilute solutions should be concentrated before applying the tests; as a rule, satisfactory results are obtained by using 0.2 c.c. of a solution containing one part of dye in 10,000.

The reagents used are as follows:

	Strength of solution %		Strength of solution %
Berberine sulphate.....	0.25	Acetic acid.....	Normal
Gold chloride.....	2.0	Potassium hydroxide.....	Normal
Phosphotungstic acid.....	10.0	Dilute ammonia.....	0.2
Silicotungstic acid.....	10.0	Chrome alum.....	5.0
Silver nitrate.....	5.0	Potassium cyanide.....	5.0
Stannous chloride.....	3.0	Wij's iodine chloride solu- tion (1/5N).....	.....
Dilute hydrochloric acid...	3.0		

The reagents used for confirmatory colour reactions are as follows:

Calcium hypochloride (sp. gr. 1.005) + 2 c.c. of glacial acetic acid per litre  
 Hydrosulphite power (10% solution)  
 Hydrosulphite B } Prepared as described by Keyes *J. Soc. Dyers & Col.*, 1927,  
 Hydrosulphite R. S. } 34, 343

The results to be expected are given in tabular form; the following is a summary of the most important:

*Picric Acid*.—Five drops (0.2 c.c.) of 1/10,000 solution gives characteristic rosettes with berberine sulphate. Potassium cyanide gives a confirmatory specific reaction—brown coloration on warming.

*Martius Yellow*.—Large yellow needle-shaped crystals, occasionally in clusters, with berberine sulphate. Distinctive needle-shaped crystals with gold chloride, and brown crystals with silver nitrate.

*Aurantia*.—Star-shaped plates are given by 0.2 c.c. of 1/10,000 solution with 1 drop of phosphotungstic acid; also with silicotungstic acid (cigar-shaped needles).

*Victoria Yellow*.—Five drops of 1/10,000 solution are boiled with 2 drops of strong hydrochloric acid, and 1 drop of Wijs' reagent, and then treated with a fragment of granulated zinc, and left for 12 to 24 hours; a pink coloration (due to dinitro-*p*-cresol) is developed.



## SUPPLEMENTARY TESTS

Tests on dilute soda solutions obtained in scheme for detection of prohibited dyes	Picric acid, trimetaphenol	Victoria yellow, dinitroresol (o- and -p)	Naphthol yellow, dinitronaphthol	Aurantia, hexanitrodi-phenylamine	Aurine, trihydroxytri-phenylmethane	Gamboge
(1) Melting point of ethereal extract from acid soln. (from literature).	121°-122.5° C.	85°-86° C., or 80.5° C.	138° C.	238° C. with decomp.	not melted at 220° C.	75°-80° C.
(2) Colour of weakly alkaline solution	yellow	orange-yellow	orange-yellow	orange-red	red	orange-red
(3) Strong in colour	yellow	yellow	yellow	orange-yellow	red	yellow
(4) Taste of soln. diluted till almost colourless	bitter	no change	no change	reddens slightly	no change	slightly turbid
(5) Strong soda added to alkaline soln	deepens considerably	no change	no change	no change	no change	soda salt thrown out as reddish-yellow ppt.
(6) Excess of salt added to alkaline soln	no change	yellow ppt.	yellow ppt.	yellow ppt.	yellow ppt.	yellow ppt. which on boiling collects as brown drops colourless
(7) Dilute acid added to alkaline soln. Strong	no change	colourless	colourless	colourless	yellow soln.	no change
(8) Weak phuric acid	no change	colourless	colourless	colourless	yellow	brownish
(9) Alk. soln. boiled with ammonium sulphide.	deep reddish-brown	faint reddish-brown	faint yellow	faint yellow	no change	no change
(10) Alk. soln. boiled with KCN	deep reddish-brown	deepens to orange brown; decolorised	brown	deep brown	slowly discoloured	no change
(11) Acid soln. boiled with SnCl <sub>2</sub>	decolorised	decolorised	decolorised	yellowish brown to deep reddish brown decolorised	no change	no change
(12) Boiled with zinc dust and ammonia	deepens to reddish brown then gradually decolorised	decolorised	decolorised	decolorised	decolorised	colour slightly fades to yellow
Re-oxidised with sodium persulphate	no change	no change	no change	no change	colour partially returns	no change
Boiled with ferrous sulphate and ammonia for one minute:	deep red	yellow	orange	brownish	practically decolorised	yellowish
Filtered filtrate:	yellow	rose pink to deep red	orange	brownish red	faint yellow	yellowish
Filtrate acidified:	yellow					

(13)	Soln. treated with lead acetate .....	no change	yellow to yellowish-orange when warmed with acetic	no change	no change	reddish-yellow ppt.
(14)	Soln. treated with zinc acetate .....	no change	yellow to yellowish-orange when warmed with acetic	no change	no change	yellow ppt.
(15)	Soln. treated with barium chloride.	no change	no change	no change	no change	reddish ppt.
(16)	Two or three drops alk. soln. dissolved in 1 ml. conc. $\text{H}_2\text{CO}_3$ cooled; few crystals $\text{NaNO}_2$ added; warmed in water bath at $80^\circ$ to $90^\circ \text{C}$ . for 1 min., then cooled in air 2 to 3 mins.	yellow	yellow	violet strong fluorescence	brownish-yellow	no change
(17)	Alk. soln. mixed with equal vol. conc. $\text{H}_2\text{SO}_4$ ; a little solid $\text{K}_2\text{MnO}_4$ added; boiled for 1 min. Few crystals of resorcinol added and boiled till water evaporated and fuming starts. Poured into soda soln.	yellow	yellow	yellow	yellow	yellow
(18)	To 1 vol. alk. soln. add 1 vol. alcohol. To 1 vol. alk. and 1 vol. 30% soda, shake:	yellow	yellow	yellow	yellow	yellow
(19)	Upper layer .....	yellow	yellow	yellow	yellow	yellow
(20)	Lower layer .....	colourless	colourless	colourless	colourless	colourless
(21)	To 1 vol. alk. soln. add 2 vols. 30% soda. Mix and stand 1 min. Add 1 vol. alcohol. 1 vol. ether, shake:	colourless	yellow	yellow	colourless	yellow
(22)	Upper layer .....	yellow	yellow	yellow	yellow	yellow
(23)	Lower layer .....	colourless	colourless	colourless	colourless	colourless
(24)	Make acid and extract with petroleum spirit. Petroleum spirit layer.	no change	no change	no change	no change	greenish-brown to black
(25)	Add few drops alcoholic $\text{FeCl}_3$ soln. to petroleum spirit layer (No. 20), shake and allow to stand.	no change	no change	no change	no change	black
(26)	To 1 vol. soln. add dil. acid till just acid, then dil. soda till just alkaline. Add 5 ml. alcohol and 2 drops saturated bromine water. Then add N/10 soda, drop by drop.	no change	no change	no change	no change	colourless, then yellow when alkaline

**Aurine.**—A few drops (0.2. c.c.) of the ammoniacal extract from the foodstuff are treated with 2 drops of chrome alum solution, and the lake formed is extracted with ether. A few drops, on spontaneous evaporation, form a pink circle, which taken in conjunction with the colour changes of aurine (yellow in acid, red in ammonia) is distinctive of aurine.

## MEAT EXTRACTS

**Definition of Extracts of Meat.**—A variety of preparations occur in commerce, under the titles of meat extract, fluid beef, beef-juice, etc. These articles, while useful and valuable in their way, do not justify the extravagant claims made respecting certain of them.

Meat extracts, meat peptones, gelatin, etc., are defined as follows by the Committee on Food Standards of the Association of Official Agricultural Chemists (October, 1907):

1. *Meat extract* is the product obtained by extracting fresh meat with boiling water and concentrating the liquid portion by evaporation after the removal of fat, and contains not less than 75% of total solids, of which not over 27% is ash, and not over 12% is sodium chloride (calculated from the total chlorine present), not over 0.6% is fat, and not less than 8% is nitrogen. The nitrogenous compounds contain not less than 40% of meat bases, and not less than 10% of creatine and creatinine.

2. *Fluid meat extract* is identical with meat extract, except that it is concentrated to a lower degree, and contains not more than 75 and not less than 50% of total solids.

3. *Bone extract* is the product obtained by extracting clean, fresh, trimmed bones of animals in good health at the time of slaughter with boiling water and concentrating the liquid portions by evaporation, after removal of fat, and contains not less than 75% of total solids.

4. *Fluid bone extract* is identical with bone extract, except that it is concentrated to a lower degree, and contains not more than 75 and not less than 50% of total solids.

5. *Meat juice* is the fluid portion of muscle fibre, obtained by pressure or otherwise, and may be concentrated by evaporation at a temperature below the coagulating point of the soluble proteins. The solids contain not more than 15% of ash, not more than 2.5%

of sodium chloride (calculated from the total chlorine present) not more than 4 nor less than 2% of phosphoric acid ( $P_2O_5$ ), and not less than 12% of nitrogen. The nitrogenous substances contain not less than 35% of coagulable proteins, and not more than 40% of meat bases.

6. *Peptones* are products prepared by the digestion of protein material by means of enzymes or otherwise, and contain not less than 90% of proteoses and peptones.

7. *Gelatin (edible gelatin)* is the purified, dried, inodorous product of the hydrolysis, by treatment with boiling water, of certain tissues, as skin, ligaments, and bones, from sound animals, and contains not more than 2% of ash and not less than 15% of nitrogen.

The following are the requisites for a meat extract, as given by Liebig (Röttger, *Lehrbuch der Nahrungsmittel-Chemie*, Leipzig, 1907, p. 135).

1. A good extract should contain no albumin and no fat (the latter not above 1.5%).
2. The water content should not exceed 21%.
3. About 60% should be soluble in 80% alcohol.
4. The nitrogen content should be from 8.5 to 9.5%.
5. The ash should vary from 15 to 25%, which, besides a little sodium chloride, consists principally of phosphates.

**Liebig's Extractum Carnis.**—The oldest preparation of the nature of a meat extract was that of Justus von Liebig. Extract of meat was first described by Proust, in 1801, but the method of manufacturing it on a commercial scale is due to Liebig, and was described by him as early as 1847. The Liebig's Extract of Meat Company was established in 1865, but the article itself has been extensively made and sold under the designation of Liebig's Extract since the year 1856. According to the original directions, the extract was to be prepared by treating lean beef (chopped fine) with 8 times its weight of cold water, straining from the insoluble fibrous matter, heating the liquid to a temperature sufficient to coagulate the dissolved albumin, filtering, and evaporating the filtrate to a syrupy condition. It is evident that both protein and gelatinoid substances are excluded from an extract prepared in the cold in the above manner. But this method of preparation was admitted by Liebig to be impracticable on a manufacturing scale, and in 1865 he stated that the only available plan was to mix the chopped flesh with water

free from gypsum, and to raise the temperature of the mixture to  $82^{\circ}$  (*Pharm. J.*, iii, 13, 414).

In the following passage, actual boiling with water is recommended by Liebig: "Those who may feel inclined to prepare extract of meat as an article of commerce, will entirely miss their aim, unless they most carefully and conscientiously seek to avoid the errors of those who have hitherto attempted it. Half an hour's boiling of the chopped meat with 8 or 10 times its weight of water suffices to dissolve all the active ingredients. The decoction must, before it is evaporated, be most carefully cleansed from all fat (which would become rancid), and the evaporation must be conducted in the water-bath. True extract of meat is never hard and brittle, but soft, and it strongly attracts moisture from the atmosphere" (Liebig's *Letters on Chemistry*).

Liebig himself has stated that 34 pounds of meat are required to produce 1 pound of extract, a fact which shows how completely the real nutritive portion of the meat must be excluded. In short, an extract of meat prepared according to Liebig's original directions is practically free from albumin, gelatin, and fat, and may be said to comprise the saline and extractive matters of the meat. Various recent analyses of the extract of meat manufactured by the Liebig Company show that the commercial preparation contains material quantities of gelatinoid substances and soluble, non-coagulable proteins. Among these extractives, creatinine, lactic acid, phosphates, and potassium salts occupy a prominent position. The true nature and value of Liebig's extract is now becoming generally recognised. Though not strictly of alimentary value, it possesses marked stimulant and restorative properties, which render it useful in exhausted states of the system. Like tea and coffee, it is a food-adjunct rather than a true food. Being rich in the flavouring matter of cooked meat ("osmazome"), Liebig's extract is often used for flavouring soups.

In a letter to *The Times* (October 1, 1872) Liebig wrote: "Neither tea nor extract of meat is nutriment in the ordinary sense; they possess a far higher importance by certain medicinal properties of a peculiar kind. The physician does not employ them as specific remedies. They serve the healthy man for the preservation of his health. Taken in proper proportions they strengthen the internal resistance of the body to the most various external injurious

influences, which combine to disturb the general vital processes, and adjust these latter . . . It is surely a grave offence against all the laws of physiology to compare tea, coffee, and extract of meat with the more common articles of food, and, because they are not that, to draw the inference, as Dr. Edward Smith has done, that they are nothing at all . . . Extract of meat is beef-tea made from fresh meat—not roasted—in the purest state, condensed to the consistency of a thick honey, to which nothing whatever is added by the manufacturer. The assertion that common salt is added to the extract is an unjustifiable invention . . . The necessity for the consumption of meat is considerably lessened when extract of meat is added to the vegetable food; in addition to the nutritive value which vegetables possess in themselves, they acquire in the soluble component parts of meat those substances which give a meat-diet its peculiar effect.”

Kemmerich failed to keep animals alive on a diet of meat extract, and the urine contained an abnormal proportion of carbon. It is not clear, however, that sufficient extract was ingested to correspond to ordinary food in the carbon and nitrogen content. M. Rubner found that the urine of dogs fed on Liebig's preparation acquired, on concentration, the peculiar odour of meat extract. He concludes that the meat extract does not contribute to the bodily heat, that the waste of tissue is neither hastened nor retarded by it, and that it passes away unaltered in composition (abst. *J. Chem. Soc.*, 1885, 409).

Druitt (*Trans. Obstetr. Soc.*, 1861, p. 143), in describing the characters of a liquid essence of beef which had been prepared according to his instructions, states that it exerted a rapid and remarkable stimulating action on the brain, and proposed it as an auxiliary to, and partial substitute for, brandy in all cases of great exhaustion or weakness attended with cerebral depression or despondency. Similar stimulating effects have been observed as resulting from a copious employment of Liebig's extract. The effect of a feast of animal food on savages whose customary diet was almost exclusively vegetable has been observed to be similar to the administration of an intoxicating spirit or drug.

**Commercial Meat Extracts.**—A great number of preparations having the general nature of Liebig's extract of meat are now articles of commerce. Some of these have received additions of

gelatin, blood albumin, meat fibre, etc., while in certain cases the albumin has been more or less peptonised. The term "Liebig's extract" has now a wide significance and has been decided by High Court of Justice to be public property. Hence, it does not always imply an article manufactured by the Liebig's Extract of Meat Company.

An interesting light on the methods of manufacturing meat extracts and pseudo-peptones is afforded by the following process, which forms the subject of a patent by Etienne and Delhaye (British Patent, 1890, No. 10, 961). After removing the tendons and grease, the meat is chopped and mashed, mixed with about half its weight of water, and heated by steam under pressure for 1 hour to a temperature ranging between 150° and 175°. A portion of the albuminoid matter is rendered soluble and goes into solution with the extractives. The residue, when pressed, forms a friable mass amounting to about one-third of the fresh meat used. This residue is treated on the water-bath with an equal weight of concentrated hydrochloric acid, until the fibromuscular tissue is quite disintegrated and decomposed, when the liquid is filtered. The insoluble residue is sold as manure. The liquors are neutralised with sodium carbonate, and then contain "peptone" and sodium chloride in solution. If pure "peptone" is required, the liquid is decolorised with animal charcoal, and dialysed to remove the salts; but if only a meat extract is required, the liquors from the steam treatment of the meat and the neutralised liquors from the acid treatment are mixed and evaporated in a vacuum until sufficiently concentrated.

It is claimed on behalf of these preparations that the various additions and methods of treatment give them value as real foods, but this is true in but a very limited sense, since the amount of such preparations which would require to be taken to furnish the carbon and nitrogen requisite to support life is enormously beyond the quantity of any of the preparations which could be consumed without upsetting the system, to say nothing of the extravagant cost of all such preparations if used in quantity necessary to sustain life.

In judging of the amount of credence to be attached to statements on the nutritive value and concentration of meat extracts and similar preparations, it should be borne in mind that fresh lean meat contains about 20% of nutritive matter and 75% of water. Hence

by the desiccation of 4 pounds weight there will be obtained 1 pound of dry substance, of which 80% is nutritive protein matter, the remaining 20% consisting of fat, meat bases, salts, etc. By no possible means can further material concentration of the nutritive matter be effected. Statements that meat extracts, meat essences, fluid meats, etc., contain the nutritive matter of 30, 40, or 50 times their weight of fresh meat are unjustifiable. Preparations still containing nearly half their weight of water, but of which a tablespoonful is said to be equal in nutritive value to a full meal of fresh lean meat, and meat lozenges and tablets weighing less than 1 grm., 1 or 2 of which are alleged to suffice for a meal, are evidently quite inefficient for their pretended purpose as concentrated forms of food.

It appears, therefore, that meat extracts have a true value as stimulants and restoratives, the proportions of meat bases, extractives, and salts present being an index of their value in this respect. On the other hand, all attempts to give them the characters of true nutritive concentrated foods can meet with but a very limited success.

A failure to appreciate these facts has caused very delusive values to be placed on such preparations, and the errors have been further magnified by the discordant methods of judging of the value of such articles. Thus Stutzer has expressed the opinion that the albumoses and peptones are the only constituents of value in a meat extract, and he ignores any meat fibre, gelatin, or coagulable albumin which may be present. Another well-known analyst regards the matters precipitated by alcohol as being the only constituents of value; but such a contention is clearly untenable, since the precipitate formed by alcohol contains a variable but very considerable percentage of non-nitrogenous extractives and salts. On treating an aqueous solution of Liebig's extract of meat with excess of strong alcohol, Allen obtained a precipitate weighing 31.8% of the original extract, and containing 11.7% of ash. The nitrogen in the precipitate corresponds to 10.6% of proteins, leaving 9.5 for non-nitrogenous extractive matters.

In the opinion of Allen, the following are the chief considerations on which a judgment should be formed of the value of a meat extract:

The percentage of water should first be taken into account. Thus a preparation which contains only 10% of solid matters must evi-



dently have less than half the food-value of the meat from which it is derived; and it might happen that, exclusive of the meat bases (valuable merely as stimulants) and the gelatin (an incomplete protein but useful as a supplement to cereal proteins), such a preparation contained a smaller proportion of nitrogenised organic matter than is present in ordinary beer.

It is usual in analyses of meat extracts to state the whole of the chlorine in terms of sodium chloride. This convention is not scientifically accurate, since the chlorine derived from the meat exists chiefly, if not entirely, in the form of potassium chloride, the balance being as sodium chloride, added in the form of common salt. Making an allowance of, say, 0.06% of sodium chloride for every unit per cent. of dry solid matter present in the preparation, any excess may be fairly regarded as having been added in the form of common salt. Thus, if a meat extract contain 25% of water (= 75% of solids) and 10% of chlorine in terms of sodium chloride, the allowance for natural chlorides would be 4.50% ( $= 75 \times 0.06$ ); and 5.50, that is, the difference between this figure and 10.00, will represent the added common salt of the sample. Added salt should, of course, be deducted in estimating the effective concentration of the preparation.

The bases are among the most important of the natural constituents of meat extracts, but unfortunately the existing methods for their estimation are far from satisfactory. The amount of meat bases in a preparation is often deduced from the percentage of nitrogen over and above that found to exist in other forms. Apart from the errors attendant on this indirect method of estimation, it is difficult to fix on a suitable factor for calculating the actual amount of meat bases from the nitrogen ascribed to that form of combination. Stutzer adopts the factor 3.12, which would be correct if the bases were wholly creatine. Hehner prefers to use the albumin-factor (6.25) for all nitrogenous constituents of meat extracts for convenience of comparison, with the knowledge that it is too high in the case of the meat bases, but he points out that by adopting it the figure obtained (by difference) for the non-nitrogenised extractive matters is much lower and probably a better approximation to the truth than when Stutzer's factor is employed. Still, with the exception of leucine, tyrosine, and carnine, the factors for calculating the nitrogen to the bases are all lower than that for creatine.

The following are the factors corresponding to the chief bases, etc., of muscle:

Substance	Formula	Proportion of nitrogen	Factor
Creatine .....	$C_4H_9O_2N_3$	42 in 131	3.12
Creatinine .....	$C_4H_7ON_3$	42 in 113	2.69
Xanthine .....	$C_5H_4O_2N_4$	56 in 152	2.71
Xanthocreatinine .....	$C_8H_{10}ON_4$	56 in 142	2.54
Hypoxanthine .....	$C_5H_4ON_4$	56 in 137	2.44
Carnine .....	$C_7H_8O_2N_4$	56 in 196	3.50
Leucine .....	$C_6H_{12}O_2N$	14 in 131	9.36
Tyrosine .....	$C_9H_{11}O_2N$	14 in 181	12.93
Urea .....	$CH_4ON_2$	28 in 60	2.14
Uric acid .....	$C_5H_4O_2N_4$	56 in 168	3.00

The added albumin and meat fibre present in some commercial meat extracts have, of course, a true food-value, but the amount of these constituents present in such a quantity of a meat extract as is usually, or could be, taken at a time is too insignificant to give it any appreciable value as nutriment.

The same remark applies to gelatin, which is present in some preparations as a product of the hot water or super-heated steam employed for the extraction, and in other cases has been added as such. Gelatin has a limited food value and can replace complete proteins to only about two thirds of the requirements. It is useful as a supplement to cereal proteins.

The albumoses and peptones present in some meat extracts have the advantage of being readily assimilated, and, so far as they go, are desirable constituents of such preparations. It is, however, almost certainly the fact that the proportion of the peptones has been greatly over-estimated, and that some preparations in which certain analyses show a material proportion of peptones are in reality almost, if not entirely, devoid of such constituents.

**Manufacture of Commercial Meat Extracts.**—Bigelow and Cook (*U. S. Dept. Agric. Bur. Chem., Bull. 114*, 1908) describe the manufacture of meat extracts in the larger establishments as follows:

"Up to a few years ago the soup liquor obtained from meat which was parboiled in the process of preparing canned meat was entirely wasted, but this liquor is now extensively utilised in the

manufacture of extracts and preparations of meat. In preparing canned meat, pieces of meat are placed in iron baskets which are suspended in large tanks containing cold water. Steam is admitted and the meat heated about  $\frac{1}{2}$  hour (30 to 40 minutes). The liquor, which is the source of meat extracts, is pumped into triple-effect vacuum pans and heated at  $71^{\circ}$  for about 4 hours. Then the solution is transferred to a single-effect finishing kettle and heated 8 hours until the water content approximates 22%.

"A first-grade extract of beef is prepared from beef alone and is usually sold in jars. An extract of the trimmed bones, to which considerable meat adheres, is also made. The trimmings include odds and ends of meat, muscle tissue, bone, etc., and the product is a second-grade article. In preparing this extract the bones are heated, not boiled, for 30 to 40 minutes, and the liquor evaporated to the consistency of extract. The extract prepared from corned beef liquor constitutes another second-grade product. This extract has a high content of nitrates and sodium chloride. In addition, there is an extract prepared from pork and other meats, sold under the general term of meat extract. Mixtures of the various meat and bone extracts are often made. A fluid meat extract is usually a 50% solution of a solid extract.

"Assuming that beef extract contains 21.7% of water, there is obtained from 100 pounds of 'soup liquor' 1.94 pounds of commercial meat extract. These figures are high, as they are calculated from the total solids present in soup liquor. The manufacturers claim that 100 pounds of 'soup liquor' will yield 1 pound of meat extract."

The cured-meat extract made in the larger establishments from liquors in which corned beef is parboiled previous to canning naturally contains much salt as well as saltpetre and sugar. The salt content is reduced to conform to the accepted standards for meat extract in the evaporators and also by means of centrifugal machines, and the extract, when finished, conforms to the standards in the other respects also.

**Bibliography on Commercial Meat Extracts.**—The following list of papers, treating of meat extracts and commercial peptones, was compiled at Allen's request by A. R. Tankard and others. Many of the references are to abstracts of foreign papers in English journals, since these are more readily accessible than the originals:—

Year	Author	Reference	Remarks
1880	A. Stutzer.....	<i>J. Chem. Soc.</i> , <b>38</b> , 676	Estimation of "protein compounds" by cupric hydroxide
1880	M. Rubner.....	<i>J. Chem. Soc.</i> , <b>38</b> , 904; <b>40</b> , 451	Nutritive value of fluid meat
1881	C. Estcourt.....	<i>Analyst</i> , <b>6</b> , 201	Composition of meat-extracts
1881	S. Darby.....	<i>J. Chem. Soc.</i> , <b>40</b> , 450	Fluid meat
1881	T. Defresne.....	<i>Pharm. J.</i> , (3), <b>12</b> , 8	Estimation of peptones
1881	C. Tanret.....	<i>J. Chem. Soc.</i> , <b>40</b> , 832	Character of peptones
1882	A. Stutzer.....	<i>J. Chem. Soc.</i> , <b>42</b> , 1239	Precipitation of proteins by cupric hydroxide
1882	A. H. Chester.....	<i>Analyst</i> , <b>7</b> , 124	Composition of various meat extracts
1882	Justice Field..... (Judgment)	<i>Pharm. J.</i> , (3), <b>13</b> , 412	Liebig Company v. Anderson
1885	O. Hehner.....	<i>Analyst</i> , <b>10</b> , 221	Analyses of beef tea
1885	F. Szymanski.....	<i>J. Chem. Soc.</i> , <b>48</b> , 822	Characters of peptone
1885-6	A. Stutzer.....	<i>Analyst</i> , <b>10</b> , 57, 73; <i>J. Soc. Chem. Ind.</i> , <b>5</b> , 37	Analysis and composition of various meat extracts
1886	H. Weiske.....	<i>J. Chem. Soc.</i> , <b>50</b> , 1087	Peptones are not precipitated by cupric hydroxide
1886	Kühne and Chittenden	<i>J. Chem. Soc.</i> , <b>50</b> , 818	Estimation of albumose and peptone
1886	S. H. C. Martin.....	<i>J. Chem. Soc.</i> , <b>50</b> , 636	Separation of peptones from other proteins by ammonium sulphate
1888	E. Schumacher-Kopp..	<i>J. Soc. Chem. Ind.</i> , <b>7</b> , 130	Analyses of Maggi's meat preparations
1888	J. König .....	<i>J. Soc. Chem. Ind.</i> , <b>7</b> , 449	Valuation of peptones
1889	König and Kisch.....	<i>J. Chem. Soc.</i> , <b>56</b> , 803	Estimation of albumose and peptone
1890	G. Bruylants.....	<i>J. Chem. Soc.</i> , <b>58</b> , 1351	Analysis of peptones
1890	A. Denaeyer.....	<i>J. Chem. Soc.</i> , <b>58</b> , 1351 <i>Analyst</i> , <b>15</b> , 101	Analysis of peptones
1891	A. Denaeyer.....	<i>Analyst</i> , <b>16</b> , 08, 234	Analysis of peptones
1891	Etienne and Delhay..	English Patent, 10, 961, 1890	Improvements in preparation of peptonised soluble meat and peptone
1892	Heaton and Vasey.....	<i>Analyst</i> , <b>17</b> , 28; <i>J. Chem. Soc.</i> , <b>62</b> , ii, 1535	Analysis of peptones and review of literature
1892-3	S. Riva-Rocci.....	<i>J. Chem. Soc.</i> , <b>62</b> , ii, 1136; <i>Chem. News</i> , <b>67</b> , 254	Estimation of albumose and peptone in stomach contents
1892-3	L. A. Hallopeau.....	<i>Pharm. J.</i> , (3), <b>23</b> , 181; <i>J. Chem. Soc.</i> , <b>64</b> , ii, 104	Estimation of peptones by precipitation with mercuric nitrate
1893	A. Stutzer.....	<i>J. Chem. Soc.</i> , <b>64</b> , ii, 146	Estimation of nitrogenous constituents of commercial peptones
1893	W. Kühne.....	<i>J. Chem. Soc.</i> , <b>64</b> , i 233	Characters of albumoses and peptones

Year	Author	Reference	Remarks
1894	E. Kemmerich.....	<i>J. Chem. Soc.</i> , 66, ii. 150	Composition of South American meat extract and meat peptone
1895	E. O. Beckmann.....	<i>Analyst</i> , 20, 44; <i>J. Chem. Soc.</i> , 68, ii. 543	Estimation of gelatin and albumin in peptone
1895	L. Hugounenq.....	<i>Analyst</i> , 20, 94	Analyses of adulterated peptones
1895	A. Stutzer.....	<i>Analyst</i> , 20, 182; <i>J. Chem. Soc.</i> , 68, ii. 543	Composition of various meat extracts
1895	A. Stutzer.....	<i>Analyst</i> , 20, 246; <i>J. Soc. Chem. Ind.</i> , 14, 897	Nitrogenous constituents of meat extracts and commercial peptones
1895	A. Stutzer.....	<i>J. Chem. Soc.</i> , 68, ii. 468	Assay of peptones by precipitation with potassium-bismuth iodide
1896	König and Bömer.....	<i>Analyst</i> , 21, 17; <i>J. Chem. Soc.</i> , 70, ii. 82	Composition of various meat extracts
1896	A. Bömer.....	<i>Analyst</i> , 21, 16; <i>J. Chem. Soc.</i> , 70, ii. 83	Precipitation of albumoses by zinc sulphate
1896	A. Stutzer.....	<i>Analyst</i> , 21, 19; <i>J. Chem. Soc.</i> , 70, ii. 84	Estimation of gelatin in meat extracts and peptones
1896	L. de Koningh.....	<i>J. Chem. Soc.</i> , 70, ii. 552	Estimation of solids in beef tea
1897	G. Bruylants.....	<i>J. Soc. Chem. Ind.</i> , 16, 640	Analysis of meat extracts
1897	A. Denaeyer.....	<i>Pharm. J.</i> , (4), 1897, ii. 3	Value of peptones
1897	Rideal and Stewart...	<i>Analyst</i> , 22, 231	Precipitation of proteins by chlorine
1897	Allen and Searle.....	<i>Analyst</i> , 22, 258	Precipitation of proteins by bromine
1897	H. Schjerning.....	<i>Z. anal. Chem.</i> , 1897, 643	Precipitants of proteins
1900	H. Schjerning.....	<i>Z. anal. Chem.</i> , 1900, 39, 545	Tannin-salt method
1905	O. Folin.....	<i>Amer. J. Physiol.</i> , 1905, 13, 48	Estimation of creatinine
1905	O. Folin.....	<i>Z. physiol. Chem.</i> , 1904, 41, 223	Estimation of creatinine
1906	Bigelow and Cook.....	<i>U. S. Dept. Agric. Bur. Chem. Bull.</i> 73	Estimation of peptones
1906	Bigelow and Cook.....	<i>J. Amer. Chem. Soc.</i> , 1906, 28, 1485	Estimation of peptones
1907	Grindley and Woods...	<i>J. Biol. Chem.</i> , 1907, 2, 309	Estimation of creatinine
1907	Emmet and Grindley...	<i>J. Biol. Chem.</i> , 1907, 2, 491	Estimation of creatinine
1908	Bigelow and Cook.....	<i>U. S. Dept. Agric. Bur. Chem., Bull.</i> 114	Composition of meat extracts
1908	P. Street.....	<i>Conn. Agr. Expt. Sta. Rept.</i> , 1908, 606-672	Composition of meat extracts
1909	A. Lowenstein.....	<i>J. Ind. Eng. Chem.</i> , 1909, 1, 252	Estimation of moisture in meat extracts
1919	Emery and Henley.....	<i>J. Agr. Research</i> , 1919, 17, 1	Composition and identification of meat extracts

ANALYSES OF CHIEF COMMERCIAL MEAT EXTRACTS (HEHNER)

Number	Description	Water	Rat (petroleum extract)	Gelatin	Albumin	Meat fibre and coagulated albumin	Albumoses	Peptones	Meat bases	Ash	Difference	Sodium chloride	Phosphoric acid	Total nitrogen
1	Liebig Company's <i>Extractum Carnis</i> .....	15.26	0.34	5.18	.....	2.12	2.01	8.06	39.32	23.51	4.20	5.81	6.97	9.07
2	Armour's Extract of Meat.....	15.97	0.21	3.31	.....	.....	1.75	5.13	41.12	29.36	3.15	9.74	6.76	8.21
3	Brand & Co.'s <i>Extractum Carnis</i> .....	17.85	0.38	4.56	.....	1.81	4.19	10.16	38.90	18.80	2.87	3.31	5.16	9.86
4	Liebig's Extract (Bovril & Co.'s make).....	22.24	0.29	5.50	.....	1.30	3.62	8.44	38.58	20.45	-0.42	5.14	5.50	9.19
5	Brand & Co.'s Meat-juice	55.48	0.10	0.69	1.00	.....	1.06	2.50	12.50	11.06	15.61	4.43	1.52	2.84
6	Valentine's Meat juice.....	55.53	0.10	0.75	0.25	.....	2.00	2.87	12.48	12.01	14.01	2.35	2.85	2.92
7	Wyeth's Meat juice.....	61.61	0.08	1.12	5.62	.....	1.08	1.86	9.44	14.78	4.41	6.96	3.01	3.06
8	Borthwick's Bouillon.....	36.19	0.25	1.37	.....	4.00	1.16	11.09	24.25	17.93	3.76	6.09	3.58	6.70
9	Vitalia Meat juice.....	70.19	0.32	0.45	16.44	0.37	0.05	0.37	2.82	8.05	2.34	5.11	0.37	3.28
10	Brand & Co.'s Essence of Beef	89.68	0.06	5.12	.....	.....	0.19	0.57	3.43	1.00	-0.05	0.33	0.40	1.49
11	Bovril Company's Fluid Beef	28.34	1.02	3.81	.....	5.37	8.38	13.18	19.38	17.67	2.85	9.07	4.05	8.02
12	Bovril Fluid Beef (unseasoned)	44.75	0.62	1.06	.....	7.31	2.38	6.25	17.12	19.90	0.61	11.42	3.34	5.46
13	Bovril for Invalids.....	24.34	1.07	4.56	.....	5.87	5.56	6.44	34.07	16.50	1.59	5.23	3.35	9.20
14	Bovril for Invalids.....	17.47	0.51	2.56	4.43	15.25	1.06	8.32	31.89	16.30	1.91	2.46	1.43	10.21
15	Caffyn's <i>Liquor Carnis</i> .....	48.46	0.11	0.25	2.19	0.94	3.05	0.98	11.30	9.95	22.17	4.43	0.62	3.09
16	Extract of Meat with Vegetable Extract.	30.03	0.10	1.69	6.12	.....	1.74	4.85	16.97	23.47	15.05	11.56	3.02	5.02

**Composition of Commercial Extracts.**—Numerous analyses of meat extracts and allied preparations have been published, but they have little value unless the exact method of analysis has been specified, and are useless for comparison, except where the various analyses of a series have been made by the same method. It must be borne in mind that preparations bearing the same names, and produced by the same firms at different dates, are liable to considerable variations in their character.

The preceeding table (p. 475) shows the composition of the chief commercial meat extracts, according to results obtained in the years 1896 and 1897, by Otto Hehner. The analyses were made by Stutzer's method (p. 508), except that in some of the later

Num- ber	Description	Nitrogen existing as						
		Gel- atin %	Albu- min %	Meat fibre and coagu- lated albu- min %	Albu- moses %	Pep- tones %	Meat bases %	Total nitro- gen %
1	Liebig's Extractum Carnis.....	0.83	.....	0.34	0.32	1.29	6.29	9.07
2	Armour's Extract of Meat.....	0.53	.....	.....	0.28	0.82	6.58	8.21
3	Brand & Co's Extractum Carnis....	0.73	.....	0.29	0.67	1.69	6.42	9.80
4	Liebig's Extract (Bovril Co.'s make)	0.88	.....	0.21	0.58	1.35	6.17	9.19
5	Brand & Co's Meat juice.....	0.11	0.16	.....	0.17	0.40	2.00	2.84
6	Valentine's Meat juice.....	0.12	0.04	.....	0.32	0.46	1.98	2.92
7	Wyeth's Meat juice.....	0.18	0.90	.....	0.17	0.30	1.51	3.06
8	Borthwick's Bouillon.....	0.22	.....	0.64	0.19	1.77	3.88	6.70
9	Vitalia Meat juice....	0.07	2.63	0.06	0.01	0.06	0.43	3.28
10	Brand & Co's Es- sence of Beef.....	0.82	.....	.....	0.03	0.09	0.55	1.49
11	Bovril Fluid Beef.....	0.61	.....	0.86	1.34	2.11	3.10	8.02
12	Bovril Fluid Beef (unseasoned).....	0.17	.....	1.17	0.38	1.00	2.74	5.46
13	Bovril for Invalids....	0.73	.....	0.94	0.89	1.03	5.61	9.20
14	Bovril for Invalids....	0.41	0.71	2.44	0.17	1.41	5.07	10.21
15	Caffyn's Liquor Car- nis.....	0.04	0.35	0.15	0.58	0.16	1.81	3.06
16	Extract of Meat and Vegetables.....	0.27	0.98	.....	0.28	0.77	2.72	5.02

analyses the albumoses were precipitated by zinc sulphate instead of by ammonium sulphate. The peptones were precipitated by phosphotungstic acid. The various nitrogenised matters were in all cases calculated from the nitrogen found by the factor 6.25 (compare p. 288). As all the analyses and calculations were made by the same method, the results are comparable.

The table on p. 476 shows the amount of nitrogen existing in different forms in the foregoing preparations.

The foregoing preparations may be roughly classified as: concentrated meat extracts, represented by analyses 1 to 4 in the table; articles of the bouillon and "meat juice" class, represented by Nos. 5 to 10; and preparations which have received an addition in material quantity of a substance not naturally a constituent of a meat extract. Thus, the finished preparations of the Bovril Company contain a variable percentage of finely divided meat fibre and sometimes added albumin. "Bovril" is stated to contain "the entire nourishment of prime ox-beef." "Invalid Bovril" "differs from ordinary Bovril in being more concentrated and quite devoid of seasoning," and is described as "the most perfect form of concentrated nourishment at present known."

The following analyses of Bovril preparations are by A. Stutzer (abst. *Analyst*, 1895, 20, 182).

	Bovril fluid beef %	Bovril fluid beef (seasoned) %	Bovril for invalids %	Bovril beef jelly %	Bovril lozenges %
Water .....	29.14	44.42	28.13	89.15	9.47
Sodium chloride.....	14.12	10.72	4.57	0.26	1.63
Other salts.....	3.38	7.60	11.50	1.04	5.71
Organic matter.....	53.36	37.26	55.80	9.55	83.19
Total nitrogen.....	8.25	5.12	8.69	1.46	11.94
Meat fibre nitrogen.....	0.73	0.90	0.70	.....	0.57
Gelatin nitrogen.....	0.09	0.09	0.15	0.29	0.70

Samples 15 and 16 are other examples of meat extracts to which additions have been made.

The following are additional published analyses of preparations which appear in the table on p. 476. The analyses are somewhat wanting in detail, but are of interest as confirmations of the general



## MEAT AND MEAT PRODUCTS

	Valentine's meat juice			Wyeth's beef juice		Brand & Co.'s essence of beef		
	R. R. Tatlock %	R. H. Chittenden, 1891 %	O. Hehner, 1893 %	R. R. Tatlock %	R. H. Chittenden, 1891 %	A. Dupré, 1886 %	R. R. Tatlock %	O. Hehner, 1893 %
Water.....	51.40	60.31	55.24	56.13	57.88	80.45	90.48	91.23
Ether extract.....	0.04	0.78	4.80	Trace	0.85	Trace	Trace	0.18
Gelatin and albumin.....	7.66	0.55	0.93	11.16	0.47	5.88	4.83	3.79
Peptone.....		Albumin	1.55		Albumin			
Creatine and meat extractives.....	18.56	None	18.27	10.04	None	4.49	2.98	3.96
Non-nitrogenous extractives.....	11.96	29.15	8.08	7.23	35.08		0.47	None
Sodium chloride.....	10.38	11.30	2.62	15.44	17.52	1.25	1.24	0.45
Other mineral matters.....		4.00	8.51			0.59		0.39
Containing P <sub>2</sub> O <sub>5</sub> .....					3.94			

## ANALYSIS OF SOLID MEAT EXTRACTS BY BIGELOW AND COOK

Maker	Moisture		Mineral constituents			Acidity			Nitrogen as—										Ether extract		Undetermined		Sample	
	%	%	Chlorine as sodium chloride in ash	Total phosphoric acid	Organic phosphoric acid	Inorganic phosphoric acid	N/10 sodium hydroxide	As lactic acid	Total nitrogen	Insoluble and coagulable	Proteoses	Peptonoses	Total meat bases	Creatine and creatinine	Xanthine bases	Meat bases other than creatine and xanthine	Ammونيا	%	%	Grm.	Net weight			
Cudahy Pkg. Co.	26.50	24.06	8.54	2.40	0.35	1.94	6.67	6.01	7.30	0.37	1.65	1.57	3.56	0.87	0.38	0.30	1.30	8.66	55.8	45				
Liebig Co.	21.14	21.03	3.11	2.40	0.61	1.79	9.04	8.13	9.07	19.20	2.68	3.82	1.14	.03	2.31	0.20	1.30	5.80	57.8	45				
Armour and Co.	21.66	20.46	5.47	4.55	.49	4.06	9.35	8.42	7.66	48.20	1.90	3.05	.75	.04	2.26	.21	50.11	67	45.7	40				
Libby McNeal and Libby.	21.86	20.92	18.32	2.53	.24	2.29	5.72	5.15	6.02	.29	.77	1.33	3.20	1.01	.11	2.08	.43	53.16	11	131.4	53			
Swift and Co.	20.10	27.28	13.51	2.89	.18	2.71	4.61	4.15	6.60	.35	1.02	1.09	3.43	.81	.45	2.17	.71	43.21	04	115.2	57			
Hammond Co.	12.30	31.68	13.25	3.19	.21	2.98	7.16	6.44	6.86	.06	.86	1.48	4.21	1.24	.52	2.45	.25	43.20	61	119.9	60			

## NITROGENOUS CONSTITUENTS OF SOLID MEAT EXTRACTS (CALCULATED FROM ABOVE)

Maker	Nitrogenous substances										Nitrogenous substances expressed in terms of total nitrogen									
	Total pro- tein, %	Insoluble and coagulable protein	Proteoses	Peptonos	Total meat bases	Creatine and xanthine bases	Meat bases other than creatine, creatine, and xanthine	Ammonia	Insoluble and coagulable protein	Proteoses	Peptonos	Total meat bases	Creatine and xanthine bases	Xanthine bases	Meat bases other than creatine, creatine, and xanthine	Ammonia				
Cudahy Pkg. Co.	12.12	2.00	10.31	9.81	11.11	2.71	1.03	7.21	0.24	4.38	22.60	21.51	48.77	11.92	5.21	31.64	2.74			
Liebig Co.	30.50	1.19	12.56	10.75	11.02	3.56	.08	8.27	.45	2.09	22.10	20.55	42.21	13.57	.31	20.22	4.08			
Armour and Co.	27.51	3.00	12.03	11.86	9.52	2.34	.11	7.05	.26	6.27	26.37	24.86	39.82	6.20	.53	20.50	2.74			
Libby McNeal and Libby	14.93	1.81	4.81	8.31	9.98	3.15	.30	6.49	.52	4.82	12.19	12.09	33.16	16.78	1.83	34.55	7.14			
Swift and Co.	15.38	2.19	6.38	6.81	10.70	2.53	1.22	6.77	.86	5.30	15.43	16.53	37.07	12.27	6.82	32.88	10.76			
Hammond Co.	15.01	.39	5.38	9.25	13.14	3.87	1.41	7.64	.30	5.87	12.54	21.57	61.37	18.08	7.58	35.71	3.64			

a The sum of insoluble and coagulable proteins, proteoses, and peptonoses.

## ANALYSIS OF FLUID MEAT EXTRACTS BY BIGELOW AND COOK

Maker	Mineral constituents				Acidity		Nitrogen as—										Ether extract		Undetermined		Sample	
	Total ash	Chlorine as sodium chloride in ash	Total phosphoric acid	Organic phosphoric acid	Inorganic phosphoric acid	N/10 sodium hydroxide	As lactic acid	Total nitrogen	Insoluble and coagulable protein	Proteoses	Peptones	Total meat bases	Creatine and creatinine	Xanthine bases	Meat bases other than creatine and xanthine	Ammonia					Net weight	Price
Armour and Co.	57.75	17.23	8.27	2.32	0.26	2.96	3.46	3.11	2.85	0.04	0.34	0.70	1.66	0.38	0.23	1.05	0.11	0.09	9.75	105.7	40	
John Wyeth and Bro.	58.84	16.21	6.71	3.27	0.04	3.21	4.35	3.92	3.15	0.46	0.10	0.47	1.92	0.26	0.26	1.40	0.20	0.23	8.12	68.6	50	
Valentine Co.	57.64	10.26	1.77	3.41	0.45	2.96	5.04	4.53	3.06	0.03	0.10	0.77	1.94	0.35	0.22	1.37	0.22	0.50	15.12	69.7	75	
Armour and Co.	49.94	15.91	7.02	3.29	0.28	2.83	5.29	4.76	3.87	0.29	0.09	0.74	2.02	0.80	0.17	1.37	0.13	0.04	12.14	63.5	35	
Cudahy Pkg. Co.	55.99	16.99	8.48	2.48	0.38	2.10	5.51	4.92	3.95	0.17	0.54	0.41	2.61	0.80	0.40	1.79	0.20	0.05	6.60	73.7	35	
Cibild Co.	64.63	16.13	11.38	0.95	0.14	1.81	2.70	3.43	3.16	0.31	0.44	0.89	1.36	0.50	0.69	0.77	0.18	0.06	2.04	102.2	45	
Mosquera-Julia Food Co.	68.97	13.85	10.05	0.80	0.18	1.62	2.45	3.20	2.41	0.08	0.31	0.91	0.98	0.26	0.64	0.64	0.13	0.09	3.54	127.4	50	

a All coagulable.

b Largely insoluble.

c Partly insoluble.

## NITROGENOUS CONSTITUENTS OF FLUID MEAT EXTRACTS (CALCULATED FROM ABOVE)

Maker	Nitrogenous bodies										Nitrogenous bodies expressed in terms of total nitrogen									
	Total protein	Insoluble and coagulable proteins	Proteoses	Peptones	Total meat bases	Creatine and creatinine	Xanthine bases	Meat bases other than creatine, creatin, and xanthine	Ammonia	Insoluble and coagulable protein	Proteoses	Peptones	Total meat bases	Creatine and creatinine	Xanthine bases	Meat bases other than creatine, creatin, and xanthine	Ammonia	Total protein		
Armour and Co.	6.76	0.25	2.13	4.38	5.18	1.19	0.62	3.28	0.13	1.40	11.93	24.56	8.25	13.31	8.07	36.84	1.86	6.76		
John Wyeth and Bro.	6.45	2.88	0.51	2.94	5.09	0.81	0.71	4.37	0.24	14.08	3.17	4.92	66.98	18.25	8.75	44.44	0.35	6.45		
Valentine Co.	5.03	1.89	4.31	4.81	6.05	1.09	0.60	4.27	0.76	7.98	7.53	12.32	20.11	4.40	7.19	44.47	7.19	5.03		
Armour and Co.	10.75	1.81	4.31	4.03	6.30	1.30	0.40	5.28	0.24	7.19	13.67	10.38	20.12	4.40	4.39	35.40	3.36	10.75		
Cudahy Pkg. Co.	7.06	1.06	3.38	5.26	4.24	1.20	0.11	1.58	0.22	9.75	13.84	27.99	42.77	15.72	2.83	24.21	5.06	7.06		
Wm. H. Bald Co.	8.25	1.94	2.75	5.69	4.24	1.00	0.24	2.40	0.22	9.75	13.84	27.99	42.77	15.72	2.83	24.21	5.06	8.25		
Mosquera-Julia Food Co.	8.13	0.30	1.94	5.69	3.06	0.81	0.22	2.00	0.16	3.32	13.86	37.76	40.66	10.79	3.32	26.56	5.39	8.13		

character of the articles in question, and as illustrating their variation from time to time.

Tatlock's and Dupré's analyses were probably made by some modification of the alcohol process (pages 490, 514). The figures of R. H. Chittenden are from his address to the Philadelphia County Medical Association (May, 1891). The results show generally that the preparations contain a large percentage of water, and that, although of value as stimulants or food-adjuncts, they cannot be regarded as concentrated forms of nutrient food.

# MEAT JUICES PREPARED IN LABORATORY (BIGELOW AND COOK)

Serial No.	Preparation of juice	Composition of sample					
		Water in juice	Ash	Chlorine as sodium chloride in ash	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	Ether extract	Acidity as lactic acid
		%	%	%	%	%	%
17091	Round beef, cold pressed....	85.76	1.53	0.12	0.37	0.27	0.27
17092	Chuck beef, cold pressed....	86.85	1.86	.20	.31	.30	.32
17091	Round beef pressed at 60°...	90.65	1.36	.15	.36	.19	.15
17092	Chuck beef pressed at 60°...	91.90	1.29	.19	.29	.64	.20
19766	Juice from beef chuck at 60°...	89.56	1.27	.16	.37	.....	.....
19767	Juice pressed from sirloin steak and water.	91.10	1.40	.12	.18	.....	.....
19785	Juice extracted from sirloin steak by cold pressure.	96.13	.46	.05	.14	.....	.....
19786	Juice extracted from beef chuck by cold pressure.	96.58	.43	.05	.11	.....	.....
19787	Juice extracted from beef chuck by cold pressure after 6 hours at 60°-100°.	98.11	.39	.05	.12	.....	.....

Serial No.	Preparation of juice	Composition of sample						
		Total nitrogen	Insoluble nitrogen	Coagulable nitrogen	Proteose nitrogen	Peptone nitrogen	Amino nitrogen	Undetermined matter
		%	%	%	%	%	%	%
17091	Round beef, cold pressed....	2.08	0.16	1.77	0.06	0.16	0.33	0.47
17092	Chuck beef, cold pressed....	1.74	.29	.98	.67	.11	.29	1.03
17091	Round beef pressed at 60°....	1.16	.....	.68	.04	.01	.43	1.90
17092	Chuck beef pressed at 60°....	1.09	.12	.41	.67	.21	.27	.40
19766	Juice from beef chuck at 60°...	1.09	.....	.49	.42	.....	.18	2.92
19767	Juice pressed from sirloin steak and water.	1.18	.....	.54	.20	.18	.26	.94
19785	Juice extracted from sirloin steak by cold pressure.	.48	.34	.....	Trace	None	.14	.85
19786	Juice extracted from beef chuck by cold pressure.	.43	.34	.....	Trace	None	.09	.59
19787	Juice extracted from beef chuck by cold pressure after 6 hours at 60°-100°.	.24	.....	0	Trace	.12	.08	.25

**MEAT JUICES PREPARED IN LABORATORY (BIGELOW AND COOK)**  
(Continued)

Serial No.	Preparation of juice	Results in terms of total nitrogen					Nitrogenous bodies				
		Insoluble protein	Coagulable protein	Albumoses	Peptones	Amino bodies	Insoluble protein	Coagulable protein	Proteoses	Peptones	Amino substances
		%	%	%	%	%	%	%	%	%	%
17091	Round beef, cold pressed	7.69	65.87	2.88	7.69	15.87	1.00	8.56	0.38	1.00	1.03
17092	Chuck beef, cold pressed	16.66	56.32	4.02	6.32	16.66	1.81	6.13	.44	.69	.90
17091	Round beef pressed at 60°	58.62		3.45	.86	37.07	4.25	.25	.06	1.34	
17092	Chuck beef pressed at 60°	11.01	37.61	6.42	19.26	24.77	.75	2.56	.44	1.31	.84
19766	Juice from beef chuck at 60°	44.95		38.53		16.51	3.06	2.63			.56
19767	Juice pressed from sirloin steak and water	45.76		16.95	15.25	22.03	3.38	1.25	1.13	.81	
19785	Juice extracted from sirloin steak by cold pressure	70.83				29.17	2.13	Trace	None	.44	
19786	Juice extracted from beef chuck by cold pressure	79.07				20.93	2.13	Trace	None	.28	
19787	Juice extracted from beef chuck by cold pressure after 6 hours at 60°-100°	0			50.00	33.33	0	Trace	.75	.25	

The "Perfected Wyeth Beef-juice" is stated by the manufacturers to contain "not only the haemoglobin but also the valuable nutritive albuminous elements of beef active and unchanged. It is carried to a very high degree of concentration, each tablespoonful containing the nutrient and stimulating principles of three-quarters of a pound of fresh lean beef. It contains many times more pure serum-albumin than any of the ordinary preparations of this class, and it does not owe any of its nitrogenous material to added egg-albumin." An analysis quoted by the manufacturers states the preparation to contain: moisture, 44.87%; organic matter (including 4.57 of nitrogen), 38.01%; and mineral matter, 17.12%.

Among the more recent analyses of meat extracts and similar preparations, those by Bigelow and Cook (*U. S. Dept. Agr. Bur. Chem. Bull.* 114, 1908) are important, and are reproduced in the preceding tables. These analyses represent samples on the market in the winter of 1905-1906.

An extensive investigation of commercial meat extracts has been made by Street (*Conn. Agr. Expt. Stat. Rept.*, 1908, 606-672), who gives a very complete bibliography. The following are Street's maximum and minimum values for thirty-five brands of paste and fluid preparations:

	Paste preparations		Fluid preparations	
	Max. %	Min. %	Max. %	Min. %
Water.....	36.54	14.79	68.37	42.03
Organic matter.....	77.90	51.56	41.07	19.05
Ash.....	36.28	14.45	21.56	11.28
Petroleum spirit extract.....	0.50	0.04	0.62	0.00
Chlorine.....	17.81	2.50	11.44	2.99
= added salt <sup>1</sup> .....	25.05	0.00	15.84	2.02
Phosphoric acid.....	6.22	1.15	2.87	0.88
Potash.....	12.65	2.29	4.81	1.73
Acidity, <sup>2</sup> phenolphthalein.....	14.50	3.74	6.64	2.40
Acidity, <sup>2</sup> litmus.....	9.07	1.90	4.24	1.18
Nitrogen, total.....	10.47	5.02	5.36	1.78
Nitrogen, insoluble.....	0.34	0.00	0.99	0.00
Nitrogen, coagulable.....	0.26	0.00	0.10	0.00
Nitrogen, ammonia.....	0.74	0.13	0.58	0.11
Nitrogen, pptd. by tannin salt.....	7.89	2.93	3.20	0.64
Nitrogen, meat bases.....	4.37	0.63	2.30	0.80
Nitrogen, pptd. by zinc sulphate.....	5.04	0.44	1.59	0.31
Nitrogen, creatinine.....	1.85	0.07	0.49	0.00
Nitrogen, creatine.....	1.30	0.03	0.48	0.90
Nitrogen, purine.....	0.83	0.16	0.36	0.07
Nitrogen, undetermined meat bases.....	1.87	0.14	1.76	0.36

<sup>1</sup> See *Allen's Comm. Org. Anal.*, 1913, Vol. VIII, 394.

<sup>2</sup> cc. N/10 KOH per grm. of sample.

Street also reports (*loc. cit.*) the analyses of certain proprietary meat preparations, as shown in the accompanying table.

Micko (*Z. Nahr. Genussm.*, 1913, 26, 321; 1914, 28, 489) has continued his extensive studies on meat extracts and bouillon cubes, and in the papers referred to discusses exhaustively the methods of analysis and the basis of interpretation of the results secured.

Einbeck (*Z. physiol. Chem.*, 1913, 87, 145) has isolated from meat extract succinic acid and fumaric acid (*Id.*, 1914, 90, 301), but could not establish the presence of malic acid. Krimberg and Izraily (*Id.*, 1913, 88, 324) isolated creatinine.

Salkowski (*Biochem. Z.*, 1913, 55, 254) points out that zinc chloride and sodium carbonate precipitate purine bases, but from

## ANALYSES OF PROPRIETARY MEAT PREPARATIONS—STREET

Brand	Water	Alcohol by weight	Organic matter	Ash	Petroleum spirit extract	Chlorine	Phosphoric acid	Potash	Acidity, c.c. N/10 KOH per gram of material		Forms of nitrogen							Meat bases			Undetermined
									Phenol-phthalein	Litmus	Total	Insoluble	Coagulable	Ammonia	Ppt. by tannin-salt	Meat bases	Ppt. by zinc sulphate	Creatinine	Creatine	Purines	
Bovine.....	68.21	6.33	30.18	1.61	0.09	0.74	tr.	0.18	0.38	0.34	2.57	0.06	0.14	0.04	2.26	0.07	2.39	0.00	0.00	0.03	0.04
Mason's essence of beef.....	88.18	0.00	10.46	1.30	0.09	0.14	0.42	0.62	0.98	0.35	1.30	0.05	0.00	0.00	0.85	0.34	0.77	0.08	0.05	0.05	0.16
Valentine's meat juice.....	55.72	0.00	32.75	11.53	0.10	0.00	2.07	5.18	6.92	4.34	3.13	0.05	0.00	0.26	0.90	1.92	0.12	0.22	0.13	0.37	1.20
Wyeth's beef juice.....	53.81	0.00	29.02	17.17	0.02	4.75	3.06	4.48	6.60	4.72	3.25	0.12	0.41	0.19	0.85	1.08	0.20	0.18	0.11	0.16	1.23
Liquid peptonoids.....	68.43	13.05	30.66	0.91	0.00	0.22	0.12	0.18	0.76	0.30	0.83	0.00	0.00	0.05	0.58	0.20	0.27	0.02	0.03	0.00	0.15
Pepito-mangan "Gude".....	81.17	13.25	17.98	0.85	0.02	tr.	tr.	0.02	0.26	0.17	0.16	0.00	0.02	0.12	0.07	tr.	0.04	0.00	tr.	0.00	0.00
Golden's liquid beef No. 2.....	68.85	16.53	30.98	0.17	0.04	0.04	tr.	0.09	0.20	0.11	0.05	0.00	0.00	0.00	.....	.....	.....	.....	.....	.....	.....
Panopepton.....	61.82	14.19	37.04	1.14	0.00	0.31	0.18	0.33	2.02	1.08	1.12	0.00	0.00	0.04	0.72	0.36	0.29	0.03	0.06	0.03	0.24
Mulford's predigested beef.....	74.30	12.52	25.32	0.38	0.00	0.26	0.02	0.10	1.44	0.80	0.50	0.00	0.00	0.00	0.31	0.19	0.13	0.02	0.02	0.03	0.12
Murdock's liquid food.....	73.53	8.60	25.75	0.72	0.30	0.19	0.04	0.14	1.10	0.28	1.98	0.39	0.00	0.13	1.18	0.28	1.28	0.00	0.00	0.00	0.28
Asparox.....	68.37	0.00	17.12	14.51	0.02	6.10	0.06	2.27	2.40	1.64	1.78	0.10	0.01	0.11	0.04	0.92	0.38	0.16	0.13	0.14	0.49
Vigoral.....	43.15	0.00	41.14	15.71	0.19	3.54	2.85	3.34	6.04	3.84	4.09	0.26	0.07	0.26	1.53	1.97	0.55	0.40	0.15	0.31	1.05
Bovox.....	51.74	0.00	30.25	18.01	0.29	8.53	0.88	1.76	3.04	1.18	4.02	0.06	0.00	0.16	2.80	1.00	1.58	0.19	0.10	0.07	0.04
Bovril.....	43.12	0.00	41.07	15.81	0.62	4.78	2.48	3.55	5.20	3.10	5.36	0.99	0.04	0.45	2.33	1.55	1.33	0.49	0.30	0.35	0.41
Cibbl's fluid extract.....	64.21	0.00	19.05	15.84	0.00	7.61	1.38	1.98	2.97	2.00	2.79	0.14	0.00	0.18	1.40	1.07	0.60	0.28	0.18	0.17	0.41
Maggi's bouillon.....	49.34	0.00	29.32	21.34	0.00	11.44	0.11	0.64	4.32	2.28	2.88	0.00	0.00	0.18	0.82	1.88	0.33	0.05	0.01	0.06	1.76
Sinnox.....	57.33	0.00	26.76	15.91	0.00	7.10	1.36	2.37	4.68	3.28	2.28	0.00	0.00	0.58	0.83	0.87	0.57	0.29	0.04	0.18	0.36
Sinnox for invalids.....	51.57	0.00	20.51	18.92	0.16	7.55	2.18	3.48	5.92	3.50	2.70	0.00	0.05	0.28	0.95	1.42	0.31	0.42	0.22	0.20	0.52
Somatosse.....	11.10	0.00	82.72	6.09	0.00	0.01	4.07	0.23	1.70	1.50	12.04	0.00	0.05	0.14	12.19	0.26	10.00	0.00	tr.	0.04	0.22
Mosquera beef meal.....	12.58	0.00	82.23	5.19	8.50	0.60	1.45	2.24	6.10	2.30	12.25	0.73	0.00	0.26	4.15	1.11	0.63	0.17	0.11	0.04	0.79

flesh extracts colloids which interfere with further isolation of the bases may also be precipitated. The colloids may be destroyed by heating the extract with dilute nitric acid before the zinc chloride treatment.

Smorodinzew (*Z. physiol. Chem.*, 1914, 92, 214, 221) compared the yield of meat bases obtained by various sub-sulphate methods and found that treatment with a 10% mercuric solution in 5% sulphuric acid and precipitation of the filtrate, after removal of mercury, with phosphotungstic acid gave the best yields of purine bases and carnosine. The addition of sulphuric acid considerably reduced the yield of carnosine; the addition of lead salts likewise reduced the yield of carnosine and only slightly improved that of methylguanidine. Purine bases are not completely precipitated by phosphotungstic

#### THE CHEMICAL COMPOSITION OF MEAT EXTRACT—WRIGHT

Sample number:	1 %	2 %	3 %	4 %	5 %	6 %	Yeast extract %
Water. ....	16.95	14.49	15.34	17.39	15.71	17.54	27.80
Organic matter. ....	66.48	66.50	65.15	61.31	62.76	62.43	50.56
Ash. ....	16.57	19.01	19.51	21.30	21.53	20.03	21.64
Acidity as lactic acid. ....	11.20	12.60	11.10	10.00	11.60	11.00	7.60
Fat. ....	0.33	0.40	0.42	0.41	0.35	0.45	0.20
Total nitrogen. ....	8.47	8.87	8.12	7.88	8.05	8.31	4.98
Insoluble protein. ....	1.17	1.23	1.23	1.02	0.98	1.12	1.18
Coagulable protein. ....	0.38	0.68	0.64	0.42	0.47	0.46	
Proteose. ....	11.41	15.49	14.39	11.81	11.49	10.21	1.75
Peptone peptid. ....	0.80	5.40	6.27	10.69	10.99	10.45	10.00
Total meat bases. ....	12.98	14.00	12.40	11.17	11.21	13.48	3.11
Creatine and creatinine. ....	5.52	6.60	3.90	4.16	3.97	5.40	0.32
Purine bases. ....	0.86	1.24	0.46	1.26	1.84	1.03	2.51
Other meat bases. ....	7.60	6.16	8.04	5.75	5.40	7.05	5.28
Ammonia. ....	0.82	0.90	0.66	0.64	0.75	0.52	0.26
Chlorine. ....	1.87	2.52	3.07	2.68	3.05	2.71	4.25
Phosphoric anhydride. ....	5.54	6.24	5.29	5.24	5.58	4.60	.....
Potassium. ....	0.76	7.60	7.80	8.14	7.92	8.32	.....
Mineral salts:							
Insoluble. ....	1.32	1.02	0.88	1.12	0.24	0.82	.....
Iron oxide. ....	0.24	0.30	0.28	0.41	0.22	0.19	.....
Calcium oxide. ....	1.24	0.28	1.12	1.37	0.94	0.82	.....
Magnesium oxide. ....	0.27	1.34	0.76	0.88	1.04	1.13	.....
Potassium oxide. ....	40.81	39.98	39.98	38.21	36.74	41.53	.....
Sodium oxide. ....	6.52	7.24	8.10	12.12	9.25	10.32	.....
Sulphur trioxide. ....	2.24	2.76	1.92	3.04	2.08	2.40	.....
Phosphoric anhydride. ....	33.48	32.82	27.11	24.60	25.91	22.96	.....
Chlorine. ....	11.27	13.25	15.73	12.54	14.16	13.52	.....



**THE NITROGENOUS CONSTITUENTS OF MEAT EXTRACTS—  
WRIGHT, BEVIS, AND NELSON**

Composition calculated to the water-free basis

Sample number:	1 %	2 %	3 %	4 %	5 %	6 %
Organic matter.....	79.44	79.89	78.85	78.04	78.25	77.84
Salts.....	20.56	20.11	21.15	21.96	21.75	22.16
Chlorine.....	2.16	2.36	2.46	2.36	2.32	2.60
Nitrogen, total.....	10.26	10.48	10.33	10.05	10.07	10.17
Nitrogen, meat base.....	4.42	4.53	5.19	5.21	4.36	4.66
Soluble in 80% alcohol:						
Organic matter.....	47.95	49.35	49.23	47.47	50.57	50.00
Salts.....	11.14	12.10	11.56	12.68	13.05	13.23
Chlorine.....	2.04	2.34	2.40	2.33	2.29	2.55
Nitrogen.....	5.71	6.28	6.31	6.18	6.70	6.54
Nitrogen, meat base.....	3.99	4.60	5.01	4.32	4.44	4.40
Insoluble in 80% alcohol:						
Organic matter.....	31.49	30.54	29.62	30.57	27.68	27.84
Salts.....	9.42	8.01	9.59	9.28	8.70	8.93
Chlorine.....	0.12	0.02	0.06	0.03	0.03	0.05
Nitrogen.....	4.55	4.20	4.02	3.87	3.37	3.63
Nitrogen, meat base.....	0.43	0.07	0.18	0.46	0.08	0.26

**CHEMICAL COMPOSITION OF MEAT EXTRACT MADE FROM  
EDIBLE VISCERA—WRIGHT AND FORSYTH**

	Diaphragm		Heart + Dia- phragm sheep	Heart sheep	Liver sheep
	Sheep	Beef			
Water.....	18.85	14.56	17.34	15.38	24.52
Organic matter.....	61.29	65.80	65.72	66.46	61.06
Sodium chloride.....	2.81	2.46	2.38	18.16	14.42
Other mineral salts.....	17.05	17.24	14.56		
Ether extract.....	0.16	0.06	0.24	0.14	0.56
Total nitrogen.....	8.83	9.15	8.94	8.05	7.03
Nitrogen soluble in 80% alcohol.....	56.49	61.92	55.74	59.02	59.50
Nitrogen insoluble in 80% alcohol.....	24.66	23.52	26.92	25.60	15.98
Acidity as lactic.....	9.39		10.26		
Glucose.....					7.42
Copper.....					present

acid and their precipitation is apparently unaffected by lead salts or sulphuric acid.

Smorodinzew found ox flesh to contain 0.024% of purine, 0.265 of carnosine, 0.051 of methylguanidine, and 0.029% of carnitine. He also found that extract of mutton contained twice the amount of purines and nearly twice the amount of carnitine found in beef

## COMPOSITION OF MEAT EXTRACTS—EMERY AND HENLEY

Extract No.	Water % solids	Ash %	Or- ganic matter %	Non- nitro- genous organic matter %	Chlorine %	Chlorin		Phosphorus		Acidity	
						Sodium chloride (chlorine X 1.65) %	Natural chlorides (total solids X 0.06) <sup>a</sup> %	Total phos- phorus pen- toxi- de %	Inor- ganic phos- phorus pen- toxi- de %	Ratio of inor- ganic to total %	N/10 sodium hydroxide to 100 gm. of extract
											Litmus c.c.
Commercial extracts											
10. Beef spleens	23.29	76.71	20.39	56.32	24.96	3.87	6.45	3.10	2.17	0.700	A <sup>b</sup>
11. Hog spleens	19.46	80.54	23.01	57.53	20.55	3.78	6.30	5.17	3.08	0.711	A
12. Roast-beef soak water	11.70	88.30	33.64	54.66	17.49	7.11	11.85	6.40	5.84	0.902	A
13. Hog livers	19.00	81.00	20.78	60.22	36.47	4.96	8.28	4.67	2.93	0.629	295
14. Bare beef bones	16.10	83.90	29.55	54.35	17.06	5.55	9.25	5.53	4.98	0.900	A
15. Regular bone	17.00	83.00	28.62	54.28	19.05	5.40	9.00	5.14	4.50	0.904	A
16. Beef livers	18.18	81.82	21.76	60.06	39.08	4.86	8.11	5.25	3.31	0.630	695
17. Pickle	11.10	88.90	27.50	61.40	31.95	5.62	9.37	1.52	1.39	0.914	509
18. Beef hearts	21.30	78.70	24.48	54.22	22.00	4.57	7.63	4.72	4.04	0.845	A
19. Chuck and plate	19.80	80.20	23.20	57.00	19.43	3.23	5.39	6.16	5.18	0.844	493
20. Corned-beef cook liquor	17.00	83.00	23.78	59.22	22.43	5.27	8.79	1.87	1.42	0.760	197
Laboratory extracts											
21. Beef spleens	24.78	75.22	19.22	57.00	17.36	2.58	4.33	5.36	4.10	0.802	A
22. Beef spleens	28.09	71.91	16.57	55.34	16.71	1.91	3.19	5.32	4.10	0.802	A
23. Hog liver	19.75	80.25	21.36	58.80	24.67	2.50	4.17	7.75	4.45	0.575	98
24. Beef spleens	30.08	69.92	20.20	49.63	16.56	3.08	5.14	5.41	3.64	0.672	A
25. Hog liver	19.36	80.64	15.98	64.66	39.55	2.15	3.59	10.59	3.35	0.317	251
26. Beef hearts	19.91	80.09	23.24	56.85	25.05	2.66	4.44	6.52	5.67	0.884	279
27. Bones <sup>c</sup>	57.35	42.65	8.09	33.56	13.21	1.78	2.97	0.71	0.63	0.887	59
28. Bones <sup>d</sup>	44.97	55.03	18.86	36.17	10.25	5.50	9.18	0.81	0.40	0.567	26
29. Chuck and plate <sup>e</sup>	11.63	88.37	27.13	61.24	23.72	2.52	4.20	6.78	4.82	0.788	880
30. Chuck and plate <sup>f</sup>	17.21	82.79	23.48	59.31	12.14	2.03	3.39	6.11	4.82	0.788	348

<sup>a</sup> See Allen (1, p. 307).<sup>b</sup> Amphoteric.<sup>c</sup> These extracts gave a very cloudy solution, muddy like, and had an offensive soapy odour.<sup>d</sup> Cold-water extract; extracted four times with cold water, then boiled and concentrated.<sup>e</sup> Hot-water extract; placed in cold water and immediately heated to boiling.

## COMPOSITION OF MEAT EXTRACTS—EMERY AND HENLEY.—(Continued)

Extract No.	Nitrogen														
	Total %	Insol- uble %	Coag- ulable %	Am- monia %	Zinc sulphate precip- itate %	Tannic- salt precip- itate %	Pep- tone- like bodies %	Meat bases							
								Total (by differ- ence) %	Crea- tine %	Pre- formed crea- tine %	Purines % Un- deter- mined %				
Commercial extracts															
10. Beef spleens	7.66	0.14	0.00	0.26	2.30	3.98	1.68	3.28	0.21	0.48	0.69	1.90	1.31	0.68	None
11. Hog spleens	7.56	0.00	0.06	0.20	2.01	4.05	2.04	3.16	0.18	0.44	0.80	1.74	1.21	0.59	None
12. Roast-beef soak water	7.94	0.08	0.00	0.35	0.82	3.53	2.71	3.98	0.25	2.27	0.59	0.87	6.13	0.79	None
13. Hog livers	4.85	0.01	0.05	0.09	0.91	2.60	1.69	2.10	0.16	0.47	0.52	0.95	1.29	0.50	None
14. Bare beef bones	7.95	0.10	0.00	0.23	1.13	3.98	2.85	3.64	0.37	1.92	0.58	0.67	5.18	1.16	None
15. Regular bone	7.96	0.16	0.02	0.23	1.20	3.55	2.35	4.00	0.42	2.02	0.57	0.99	5.46	1.32	None
16. Beef livers	5.40	0.03	0.06	0.09	1.70	3.02	1.32	2.20	0.23	0.46	0.51	1.00	1.26	0.73	None
17. Pickle	6.66	0.00	0.05	0.27	0.76	2.45	1.69	3.89	0.07	1.14	0.38	2.30	3.99	0.25	14.45
18. Beef hearts	7.10	0.06	0.00	0.40	1.23	2.82	2.56	3.88	0.32	1.34	0.65	1.48	3.65	1.00	None
19. Chuck and plate	8.09	0.01	0.06	0.30	1.44	3.57	2.13	4.15	0.51	1.09	0.57	1.38	4.57	1.61	None
20. Corned-beef c o k liquor	7.66	0.08	0.06	0.50	1.32	3.16	1.84	3.86	0.36	1.24	0.58	1.68	3.34	1.13	8.70
Laboratory extracts															
21. Beef spleens	7.51	0.10	0.14	0.74	1.68	4.21	2.53	3.32	0.00	0.06	0.91	1.35	0.18	0.01	None
22. Beef spleens	7.75	0.17	0.00	0.81	1.74	3.64	1.90	3.13	0.00	0.06	1.08	1.99	0.17	0.01	None
23. Hog liver	6.53	0.00	0.06	0.50	1.58	3.84	2.26	2.04	0.03	0.08	0.25	1.68	0.22	0.03	None
24. Beef spleens	6.36	0.00	0.40	0.30	1.33	3.53	2.20	2.13	0.00	0.08	0.43	1.62	0.22	0.02	None
25. Hog liver	5.26	0.04	0.07	0.24	0.60	2.75	2.15	2.16	0.01	0.07	0.29	1.79	0.19	0.03	None
26. Beef hearts	7.04	0.12	0.00	0.83	0.78	2.15	1.37	3.93	0.47	1.05	0.57	1.25	4.16	1.28	None
27. Bones	3.52	0.18	1.46	0.09	0.54	1.26	0.72	0.53	0.00	0.22	0.05	0.26	0.59	0.00	None
28. Bones	4.64	0.00	0.09	0.18	2.58	3.57	0.99	0.90	0.01	0.19	0.68	0.70	0.53	0.04	None
29. Chuck and plate	8.57	0.03	0.03	0.70	1.61	2.30	5.45	0.11	2.41	0.91	0.70	2.25	6.48	0.37	None
30. Chuck and plate	9.66	0.03	0.05	0.65	2.09	4.73	2.64	4.23	0.91	0.91	0.70	1.71	2.46	2.84	None

extract, but only one-third the carnosine and one-half the methylguanidine. Mutton also contains more purines and carnitine and less carnosine and methylguanidine than horse flesh.

The chemical composition of New Zealand meat extracts has been reported by Wright (*Trans. Proc. New Zealand Inst.*, 1910, 43, 1). Analyses were made according to methods then in use (*J. Soc. Chem. Ind.*, 1907, 1229). His results are shown in the table on p. 486.

The nitrogenous constituents of meat extracts have been studied by Wright, Bevis, and Nelson (*Trans. Proc. New Zealand Inst.*, 1921, 53, 479). The water, ash, chlorides, nitrogen, and meat bases were determined as in the previous work. The meat base nitrogen reported is the tannin-salt filtrate after deducting the ammonia nitrogen determined by the magnesium oxide distillation method. For the 80% alcohol precipitate and the soluble extract the method of Thorpe was used (*Dictionary of Applied Chemistry*, 1912, Vol. III, p. 428). The results are given in the table on p. 485.

Wright and Forsyth (*New Zealand J. Sci. Tech.*, 1926, 8, 305) report the composition of meat extracts made from edible viscera.

Emery and Henley (*J. Agric. Res.*, 1919, 17, 1) have studied meat extracts and have reported on their composition and identification. They prepared 11 commercial extracts and 10 laboratory extracts from known sources of material and examined them by methods essentially those described by Street (*Conn. Agr. Expt. Sta. Report*, 1908, 606). The results are shown in the table on p. 487.

Some of the findings of Emery and Henley are of considerable interest. They found that the total nitrogen of liver extracts is very low. Chuck and plate extracts contain the largest quantity of nitrogen, with spleen extracts next. The pickle extract is low in nitrogen. Heart extracts and liver extracts are exceptionally high in non-nitrogenous organic matter. The determination of total nitrogen and meat base nitrogen enables one to differentiate liver extracts and spleen extracts, both being low in meat base nitrogen, whilst the total nitrogen of the spleen extract is high. The greatest and most uniform differences were shown in the total creatinine content of the various extracts. Liver and spleen are low in total creatinine. Differences were found in the phosphorus content and in its distribution. An acetic acid precipitation test,

suggested by Chapin, was tried on the extracts, and it was found that only with the spleen extracts was a decided precipitate formed. The ash of the liver extracts consistently showed the presence of copper.

**Partition of Nitrogen in Meat Extracts.**—Cook (*J. Amer. Chem. Soc.*, 1914, 36, 1551) has continued his studies on means of differentiating plant, yeast, and meat extracts, using the methods previously employed, together with the following method of Rippetoe for nitrogen precipitated by acid-alcohol:

**Nitrogen Precipitated by Acid-alcohol.**—Transfer 10 c.c. of an aqueous solution of the extract (= 1 grm.) to a 200 c.c. glass-stoppered measuring cylinder, add 1.2 c.c. of 12% hydrochloric acid, mix, add absolute alcohol to the mark, mix thoroughly and set aside for several hours at 20–25°. If necessary, make up to mark, filter and transfer 100 c.c. to a Kjeldahl flask, evaporate the alcohol on a water-bath and determine the nitrogen in the residue.

Cook found no creatine or creatinine and very little purine nitrogen in the plant extracts. The yeast extracts showed high purines and no creatine or creatinine. Plant and yeast extracts did not give the biuret reaction. All of the nitrogen of the plant extract was found in the filtrate, after applying acid-alcohol, in the form chiefly of mono-amino acids and ammonia. About 25% of the

#### PARTITION OF NITROGEN OF PLANT, YEAST, AND MEAT EXTRACTS—COOK

	Percentage of total nitrogen								
	Total nitrogen	Ammonia nitrogen	Total creatinine nitrogen	Purine nitrogen	Nitrogen in phosphotungstic acid filtrate	Nitrogen in acid-alcohol filtrate	Nitrogen in tannin salt filtrate	Amino-nitrogen	
								Formol method	Van <sup>1</sup> Slyke method
Meat extracts.	9.56	2.62	22.49	3.35	7.64	78.04	54.91	10.94	18.50
	9.65	2.49	22.59	3.52	6.84	78.45	55.85	10.94	18.23
	7.68	1.56	32.42	2.86	71.35	100.00	73.04	10.63	15.63
	9.65	.....	29.01	4.92	49.74	76.73	59.37	10.27 <sup>2</sup>	15.54
	7.49	.....	27.50	0.13	.....	89.19	64.21	9.53	17.89
Yeast extract.	5.35	2.43	0	11.22	49.96	72.15	56.07	17.64	32.71
Plant extracts.	5.23	7.46	0	0.42	85.85	100.00	94.46	36.71	65.77
	6.34	12.78	0	0.44	84.08	99.69	89.95	36.43	57.41
	6.33	.....	0	0.25	.....	100.00	83.41	30.64	45.18
	6.27	11.32	0	0.44	83.25	98.56	92.66	35.88	67.78
	6.47	6.03	0	.....	63.68	100.00	84.54	30.75	44.20
	6.08	12.98	0	0.46	84.04	99.67	92.76	35.85	61.18
	6.63	10.56	0	0.23	86.88	99.25	91.40	34.08	57.31

<sup>1</sup> Not corrected for 15% of the ammonia nitrogen.

<sup>2</sup> Not corrected for ammonia nitrogen.

nitrogen of the meat and yeast extracts is precipitated by acid-alcohol. The plant extracts yield a much larger percentage of ammoniacal nitrogen than the meat or yeast extracts.

**Bouillon Cubes.**—Many analyses of these preparations have been reported and methods worked out for estimating the percentage of meat extract present (see Cook, *J. Amer. Chem. Soc.*, 1914, **36**, 1551, and Micko, *Z. Nahr. Genussm.* 1913, **26**, 321; 1914, **27**, 489).

Cook (*J. Ind. Eng. Chem.*, 1913, **5**, 989) gives the following analyses of certain typical brands:

ANALYSES OF BOUILLON CUBES—COOK

Brand	Solids %	Organic matter %	Ether ex- tract %	Ash %	Total chlorine as NaCl %	Total phosphoric acid %	Acidity (c.c. N/20 KOH per gram.)	Total nitro- gen %	Nitro- gen ppt. by acid- alcohol %	Total creati- nine %
Behrend .....	96.60	22.86	1.93	73.74	72.13	1.02	6.20	2.19	0.13	0.84
Oxo .....	95.06	25.31	3.10	69.75	65.00	1.51	6.50	2.97	0.86	1.07
Steero .....	96.05	28.41	1.20	67.64	62.15	1.83	9.15	3.62	0.76	1.67
Burnham .....	96.87	41.94	1.00	54.93	52.90	0.58	6.10	2.11	0.05	0.88
Sunbeam .....	95.73	45.23	1.44	50.50	49.26	0.54	7.30	2.36	0.02	0.92
Armour .....	96.05	26.48	0.96	69.57	67.44	0.62	6.00	2.79	0.17	1.07
Morris .....	96.77	33.00	3.79	63.77	59.17	1.69	9.68	3.67	0.56	1.07
Standard .....	95.81	21.76	4.19	74.05	72.22	0.48	5.01	2.09	0.07	0.50
Liggett .....	96.00	21.91	4.58	74.09	71.98	0.41	4.75	2.11	0.05	0.49
Knorr .....	95.44	26.44	4.57	69.20	65.00	1.55	7.40	3.20	0.91	1.38

According to Serger (*Konserven-Zeit.*, **48**, 378), if we place the creatinine content of meat extract at 6%, then bouillon cubes should contain the following amounts of creatinine for the respective amounts of meat extract used:

Meat extract used %	Creatinine contained %
25	1.5
20	1.2
15	0.9
10	0.6

Geret (*Z. Nahr. Genussm.*, 1912, **24**, 570) found from results based on the analysis of 100 samples that the meat extract of bouillon cubes varied from 20 to 25% in the best cubes to none at all in the inferior grades, calculating the solids of high-grade meat extract at 80%. The cubes contain about 70% of water, fat, and sodium chloride and 30% of any one or any combination of meat extract, plant extract and plant seasoning compounds, the last named consisting of amino-acids derived from proteins by heating with acid until no biuret reaction is given, and then neutralising with soda.

Serger (*Z. öffent. Chem.*, 1914, 20, 80, 101) claims that bouillon cubes should contain not more than 8% of water, 9% of fat, 65% of sodium chloride, and not less than 15% of meat extract. Good cubes contain between 92 and 98% of solids, 3.0 to 3.9% nitrogen, 0 to 5% of sugar, 0.9 to 1.5% of creatinine, 62 to 67% of ash, 1.2 to 1.7% of phosphoric acid, 18 to 25% of protein, and a ratio of N/P of 2.3 to 2.5.

Remy (*Pharm. Zentralh.*, 1913, 54, 1238), analysing 10 high-grade samples, found 12.3 to 17.8% of mineral matter (of which 11.3 to 16.2% was sodium chloride), 10.7 to 17% water, and 2.1 to 10.6% fat. Inferior products, containing as much as 60% of sodium chloride, have been observed.

Kapeller and Gottfried (*Z. Nahr. Genussm.*, 1913, 26, 161) found 9 samples to range from 3.4 to 7.2% of water, 4.4 to 10.9% of fat, 9.8 to 24.8% of albuminoids, 54.6 to 76.6% of ash, and 51.6 to 74.4% of sodium chloride. The same chemists analysed 8 other samples, with results as follows (*Z. Nahr. Genussm.*, 1914, 28, 224):

Water %	Albuminoids %	Fat %	Ash %	Sodium chloride %
6.5	13.4	6.7	65.9	63.3
3.3	14.6	8.2	56.3	54.9
4.4	14.1	6.2	69.1	66.2
3.4	10.1	5.7	77.2	74.5
3.8	9.6	8.3	70.4	69.4
3.3	6.6	10.5	73.1	72.5
5.6	6.0	7.0	70.6	69.1
1.1	0.5	2.9	83.7	83.0

Conti (*Boll. chim. farm.*, 1913, 51, 183) analysed 3 samples, the last consisting largely of gelatin and salt:

Water %	Organic matter %	Nitrogen %	Fat %	Ash %	Sodium chloride %	Phosphoric acid %
6.5	17.0	2.48	4.2	71.9	65.0	1.06
7.0	22.0	2.49	7.1	63.0	60.0	0.40
22.0	51.5	7.64	.....	26.5	24.0	0.58

Krasser (*Z. Nahr. Genussm.*, 1914, 27, 78) found that Maggi's bouillon cubes during the years 1908-1911 ranged from 52.4 to 56.6%

of water, 21.9 to 27.2% of organic matter, 20.0 to 25.7% of albuminoids, 20.4 to 21.6% of ash, 17.0 to 18.7% of sodium chloride, and 0.87 to 1.13% of phosphoric acid.

Street (*Conn. Agr. Expt. Stat. Rept.*, 1908, 660; 1911, 161; 1914, 238) gives the following analyses of bouillon cubes and other condensed soups:

	Water %	Fat %	Ash %	Pro- tein %	Carbo- hydrates %	Nitro- gen %	Sodium chloride %
Knorr's pea soup with bacon.....	9.6	9.0	15.0	21.4	45.0	3.42	12.5
Knorr's bean soup.....	11.0	6.2	16.0	19.3	47.5	3.09	12.8
Knorr's consommé.....	4.3	5.4	66.5	.....	.....	3.10	61.5
Steero bouillon cubes.....	6.6	1.7	65.2	.....	.....	3.89	59.5
Oxo bouillon cubes.....	4.8	3.6	67.5	.....	.....	3.28	62.7
Armour's beef extract and vegetable tablets..	10.2	0.4	29.6	.....	.....	1.63	22.4
Mason's beef tea lozenges	10.8	0.1	9.7	.....	.....	13.04 <sup>1</sup>	2.2
Anker's bouillon capsules	8.6	0.1	47.1	.....	.....	5.54	38.4

<sup>1</sup> Contained much gelatin.

**Soups.**—Street (*Conn. Agr. Expt. Stat. Rept.*, 1910, 493) analysed 6 brands of canned bouillon showing the following range of composition (over 99% of 1 brand consisting of water and salt):

	Max. %	Min. %	Av. %
Water.....	96.4	92.0	94.6
Ash.....	2.7	1.1	1.9
Ether extract.....	0.15	0.06	0.09
Protein (N × 6.25).....	2.09	0.49	1.39
Sodium chloride.....	2.48	0.93	1.63
Nitrogen.....	0.334	0.079	0.222
Undetermined.....	4.21	0.33	2.05

Congdon (*No. Dak. Food Dept. Spec. Bull.*, 1913, 2 (15), 246; 1914, 3 (5), 62) has also investigated certain condensed soups. The amount of meat fibre in chicken soup ranged from 2.18 to 16.48%, the liquid portion from 6.01 to 83.88%, and the other ingredients (chiefly boiled rice) from 13.94 to 90.32%. He showed that the average composition of 8 brands of "condensed" chicken soup was as shown in table on p. 494.

On the original basis these samples ranged from 81.4 to 94.1% water, 0.94 to 4.13% of protein (N × 6.25), 0.07 to 1.96% of water-



Boiled rice.....	27.18
Meat extract.....	3.54
Meat fibre.....	8.10
Salt.....	2.05
Water.....	59.13

soluble protein, 0.06 to 1.33% of fat, 1.17 to 12.03% of carbohydrates, 1.09 to 3.12% of ash, and 0.92 to 2.83% salt. In some instances more beef fibre was present than chicken.

## ANALYSIS OF MEAT EXTRACTS AND SIMILAR PRODUCTS

The methods of analysis of meat extracts and similar products are not dissimilar from those used in the analysis of meat or water extracts. The method of preparation differs somewhat and certain additional determinations are usually made. The following methods are those of the A. O. A. C. (*Methods of Analysis*, 1925, 250) with slight modifications by the author.

### A. O. A. C. Methods

**Preparation of Sample.**—Remove liquid and semi-liquid meat extracts and similar preparations from the container and mix thoroughly before sampling. A little heating expedites the mixing of pasty extracts. The sediment that forms in many liquid preparations should be carefully removed from the bottom of the container and included in the sample. If the sample is in the form of cubes, grind 10–12 of the cubes in a mortar.

**Moisture.**—Moisture is determined at the temperature of boiling water and under a pressure of 100 mm. of mercury, employing 2 gm. of powdered preparations, about 3 gm. of pasty preparations, and 5–10 gm. of liquid extracts, according to the solid content. The powdered preparations are dried directly without admixture. The pasty preparations are dissolved in water and dried with sufficient ignited sand, asbestos, or pumice stone to absorb the solution. When glycerin is present, the preparation is dried over sulphuric acid in a vacuum desiccator, as described on p. 282.

**Ash.**—A weighed quantity of the sample, representing about 2 gm. of dry material, is charred and then burned at a low heat, not exceeding dull redness, until free from carbon. When the proper type of container has been used, the residue from the moisture determination may be employed. If a carbon-free ash cannot be obtained in this manner, the charred mass is exhausted with hot

water, the insoluble residue collected on an ashless filter, and the filter and contents burned to a white or nearly white ash. The filtrate is added, the liquid evaporated to dryness, and the residue heated at dull redness until the ash is white or grayish white, then cooled in a desiccator, and weighed. Materials containing any considerable amounts of sodium chloride or other easily volatilised or fused salts must be treated by the charring and extracting method.

**Total Phosphorus.**—The residue from the ash determination or a residue similarly prepared is used for the determination of total phosphorus. The procedure is the same as for meat (see page 289).

**Chlorides.**—Dissolve about 1 grm. of the sample, prepared as directed under "Preparation of Sample," in 20 c.c. of 5% sodium carbonate solution, evaporate to dryness, and ignite as thoroughly as possible at a temperature not exceeding dull redness. Extract with hot water, filter, and wash. Return the residue to the dish (platinum) and ignite to an ash; dissolve in dilute nitric acid (1 + 4), filter from any insoluble residue, wash thoroughly, and add this solution to the water extract. Add a 10% silver nitrate solution, avoiding more than a slight excess. Heat to boiling, protect from the light, and allow the vessel to stand until the precipitate is granular. Filter on a weighed Gooch crucible, previously heated to 140°–150°, and wash with hot water, testing the filtrate to prove excess of silver nitrate. Dry the silver chloride at 140°–150°, cool, and weigh.

**Fat.**—Transfer the residue from the determination of moisture (or use the cone or extraction shell containing the dried residue, if that method has been used) to a continuous extraction apparatus and extract for 16 hours, using anhydrous ether. Dry the extract at the temperature of boiling water for 30 minutes, cool in a desiccator, and weigh; continue, at 30 minute intervals, this alternate drying and weighing until the weight is constant.

**Total Nitrogen.**—The determination is carried out in the same way as for meats, the size of the sample depending upon the nitrogen content. In case nitrate or nitrite is present (for example, in cured meat extracts from corned beef cooking liquors), it should be removed before the estimation of organic nitrogen is carried out by adding 10 c.c. of freshly prepared (or properly stored) saturated ferrous chloride solution and 5 c.c. of strong hydrochloric acid and boiling a sufficient length of time.

**Ammonia.**—Introduce 1 grm. of pasty extracts or 2–3 grm. of fluid extracts into the sample tube of the Folin apparatus and proceed as directed for meat.

**Insoluble Nitrogen.**—Dissolve in cold water 5 grm. of powdered preparations, 8–10 grm. of pasty extracts, and 20–25 grm. of fluid extracts. Filter, and wash with cold water. Transfer the filter paper and contents to a Kjeldahl flask and determine the nitrogen as usual, preferably by the Kjeldahl-Gunning-Arnold method. If a large quantity of insoluble matter is present, transfer the weighed sample to a volumetric flask, dilute to a definite volume, shake thoroughly, filter through a folded filter, and determine nitrogen in an aliquot part of the filtrate. Deduct the percentage of nitrogen in the total filtrate from the percentage of total nitrogen to obtain the percentage of nitrogen in the insoluble protein. It must be remembered that, if nitrates or nitrites are present, they must be removed from the aliquot part, taken as described under total nitrogen.

**Coagulable Nitrogen.**—Prepare a solution of the sample as directed under insoluble nitrogen. Employ as large an aliquot portion of the filtrate from the insoluble nitrogen as practicable, and neutralise to phenolphthalein by the addition of acetic acid or sodium hydroxide, whichever may be necessary; add 1 c.c. of 1 *N* acetic acid, boil for 2–3 minutes, cool to room temperature, dilute to 500 c.c., and pass through a folded filter. Determine the nitrogen in 50 c.c. of the filtrate. Subtract the percentage of nitrogen thus obtained from the percentage of soluble nitrogen, to obtain the percentage of coagulable nitrogen.

**Proteoses and Gelatin.**—Evaporate the filtrate from the above determination (coagulable nitrogen) to a small volume and saturate with zinc sulphate (about 85 grams to 50 c.c., avoiding such an excess as would later cause lumping). Set aside for several hours, filter, and wash the precipitate with saturated zinc sulphate solution. Place the filter and precipitate in a Kjeldahl flask and determine the nitrogen as usual. If the precipitate is voluminous which is unusual, dilute to a definite volume with saturated zinc sulphate solution; filter; and determine the nitrogen in an aliquot part of the filtrate. Subtract the nitrogen thus obtained from the nitrogen in the filtrate from the coagulable nitrogen to obtain the nitrogen of the precipitated protein (proteoses and gelatin).

**Gelatin.**—Prepare a 50% solution of the sample, using hot water. Allow the solution to cool and place it in an ice box for 2 hours. If gelatin is present the solution will set. (This, of course, does not apply to liquid preparations which are originally fluid.) The ratio of total creatinine to total nitrogen in a normal meat extract (1:1.5) assists in determining the presence of gelatin or derivatives. The ratio is decreased when gelatin or its derivatives are present in any considerable quantity.

**Amino Nitrogen.**—Amino nitrogen may be determined by the Folin or Sørensen methods. (See pp. 327, 328.)

**Acid Alcohol-soluble Nitrogen.**—Transfer 10 c.c. of an aqueous solution of the sample (10 grm. of the sample dissolved in sufficient water to make 100 c.c.) or, if the sample is insoluble in water, 1 grm. of the sample and 10 c.c. of water, to a 200 c.c. glass-stoppered measuring cylinder; add 1.2 c.c. of 12% hydrochloric acid; mix; and add absolute alcohol to the 200 c.c. mark. Mix thoroughly and set aside for several hours. If necessary, make up to volume, filter, transfer 100 c.c. of the filtrate to a Kjeldahl flask, evaporate the alcohol on a water-bath, and determine nitrogen in the residue as usual.

**Creatine.**—Dissolve about 7 grm. of the sample in cold (20° C.) ammonia-free water in a 150 c.c. beaker, transfer the solution to a 250 c.c. volumetric flask, dilute to the mark, and mix thoroughly. Transfer a 20 c.c. aliquot part of this solution to a 50 c.c. volumetric flask and proceed as directed under creatine in water extracts. Directions applicable to meat extracts are also given on p. 299.

**Creatinine.**—For creatinine in beef extract measure about 5 c.c. of the solution, prepared as described for creatine, into a 500 c.c. volumetric flask, add 10 c.c. of 10% sodium hydroxide solution and 30 c.c. of saturated picric acid solution (1.2%), mix, rotate for 30 seconds, and continue as under the A. O. A. C. directions on p. 299. The new Folin procedure is preferable to this, however. The method is described on pp. 301, 303.

**Nitrates and Nitrites.**—In meat extracts prepared from the cooking liquors of cured meats both nitrates and nitrites may be present. To determine these substances proceed as directed under the analysis of cured meats, pp. 427 and 431.

**Glycerol.**—Weigh 2 grm. of a solid or 5 grm. of a liquid preparation in a small lead dish or thin glass shell containing 20 grm. of ignited

sand. Transfer the dish and its contents to a mortar containing more ignited sand and several grm. of anhydrous sodium sulphate and mix thoroughly. Transfer the mixture, including the dish, to a Soxhlet apparatus that has a piece of cotton placed in the side arm to prevent solid particles from being siphoned over. Extract the entire mass with redistilled anhydrous acetone for 10 hours. Distil the acetone from the extract, carefully removing the last trace by means of a vacuum pump. Take up the residue in water, add 5 c.c. of 10% silver nitrate solution, dilute to a volume of 100 c.c., shake, leave over-night, filter, and determine the glycerol in an aliquot part of the filtrate. To the aliquot part in a 250 c.c. volumetric flask add 30 c.c. of strong potassium dichromate solution (74.55 grm. of dry, recrystallised potassium dichromate dissolved in water; 150 c.c. of concentrated sulphuric acid added; cooled; and diluted with water to 1 litre at 20° C. One c.c. of this solution is equivalent to 0.01 grm. of glycerol. All volumetric measurements of this solution must be made at 20° C. on account of the big coefficient of expansion of this solution). Add carefully 24 c.c. of concentrated sulphuric acid, rotating the flask gently to mix the contents, and avoid violent ebullition, and then place in a boiling water-bath for exactly 20 minutes. Remove the flask from the bath, dilute, cool, and make up to the mark at room temperature. The quantity of strong dichromate solution used must be sufficient to leave an excess of about 12.5 c.c. at the end of the oxidation, the quantity given above (30 c.c.) being sufficient for solutions containing about 0.35 grm. or less of glycerol per 100 c.c.

Standardise the ferrous ammonium sulphate solution (these solutions will be described below) against the dilute potassium dichromate solution, using potassium ferricyanide as an outside indicator. From this titration calculate the volume (F) of ferrous ammonium sulphate solution equivalent to 20 c.c. of the dilute and, therefore, to 1 c.c. of the strong dichromate solution.

Place the oxidised glycerol solution in burette and ascertain how many c.c. are equivalent to (F) c.c. of the ferrous ammonium sulphate solution and, therefore, to 1 c.c. of the strong dichromate solution. Then 250, divided by this last equivalent, equals the number of c.c. of the strong dichromate solution present in excess in the 250 c.c. flask after oxidation of the glycerol. The number of c.c. of the strong dichromate solution added, minus the

excess found after oxidation, equals the amount used up by the glycerol.

**Solutions.**—The strong potassium dichromate solution has been described above. The dilute solution is made by diluting 25 c.c. of the strong solution, measured at 20° C., to 500 c.c. with water in a volumetric flask. Twenty c.c. of this solution are equivalent to 1 c.c. of the strong solution.

The ferrous ammonium sulphate solution is prepared by dissolving 30 grm. of crystallised substance in water, adding 50 c.c. of concentrated sulphuric acid, cooling, and diluting with water to 1 litre. One c.c. of this solution is approximately equal to 1 c.c. of dilute dichromate.

Dissolve 1 grm. of crystallised potassium ferricyanide in 50 c.c. of water. This indicator solution must be freshly prepared.

In the case of solid meat and yeast extracts a blank of 0.5–1.0% is obtained in most cases.

**Sugar.**—Heat 20 grm. of the sample with about 200 c.c. of water on a steam-bath until all soluble substances have gone into solution, cool, and proceed as directed under cured meats (p. 436). Reducing sugars to the extent of 0.5% may be present as a natural constituent of meat extracts.

Smith (*J. Ind. Eng. Chem.*, 1916, 8, 1024) proposes a method of estimating sugar in meat products which is particularly adapted to meat extracts. Five grm. of meat extract in about 25 c.c. of water (or aqueous decoction from 50 grm. of finely divided meat product) are treated with an excess (4–6 grm.) of solid picric acid and an excess (40–60 c.c.) of a 20% aqueous solution of phosphotungstic acid and made up to 100 c.c. and filtered. An aliquot part (60 c.c.) of the filtrate is treated with 3 c.c. (more if necessary) of hydrochloric acid and made up to 66 c.c. and filtered rapidly, and the reducing sugars estimated without delay by means of Fehling's solution. In the presence of cane sugar the results will be accurate within 0.1 or 0.2%, provided that the hydrochloric acid in the filtrate is neutralised at once; otherwise slow inversion will continue. Another portion of the filtrate is inverted with hydrochloric acid, and the invert sugar is estimated.

**Preservatives.**—Preservatives may be determined as described on p. 440.

**Metals.**—Metals may be determined as under canned meats (p. 368).

### Other Methods

Richardson (*Allen's Commercial Organic Analysis*, 4th Ed., 1909-14, VIII, 404) states that the analysis of extracts of meat, commercial peptones, and allied preparations is both difficult and tedious, and in some respects cannot be effected satisfactorily by the then existing methods. The following processes are those which, in his experience, are the most satisfactory for their intended purpose.

**Water and Total Solid Matters** in meat extracts may be estimated by evaporating a known weight of the sample to dryness at 100° and drying till constant in weight, preferably in the vacuum oven. From 3 to 25 grm. should be employed, according to the nature of the preparation. Stutzer weighs the quantity intended for the estimation of water into a thin basin of tin-foil (about 20 mm. high and 55 mm. in diameter), dissolves it in a little hot water, and adds sand (previously ignited and freed from fine dust by a sieve) in sufficient quantity to absorb the liquid almost completely. The basin is then heated in the water-oven until the weight is constant. The weight of the tin-foil and sand being deducted, the solid matter of the extract is obtained. The tin basin and its contents are than used by Stutzer for the determination of the gelatin (p. 508).

In the case of samples containing gelatin, or which from other circumstances cannot be readily dried, L. de Koningh treats the preparation with a weighed quantity of tannin containing a known amount of dry matter. The mixture is evaporated and dried in the water-oven till constant in weight, when the weight of dry tannin is deducted from the residue obtained.

A method for estimating moisture in products of a viscous or semi-solid consistence, by the use of alcohol, which is applicable to meat extracts has been proposed by Lowenstein (*J. Ind. Eng. Chem.*, 1909, 1, 252). Two grm. or more of the sample are weighed into a small metal dish, about 2 5 in. in diameter, together with a short glass stirring rod flattened at one end to facilitate spreading the material over the bottom of the dish. About 15 c.c. of 95% or, better, absolute alcohol are added and thoroughly incorporated with the sample. The dish is then placed on the steam-bath, and the alcohol evaporated with constant stirring; another portion of 15 c.c. is then added and evaporated in a similar manner. In case the sample is very difficult to dry, 4 applications of alcohol may be made. Drying is continued on the steam-bath for 30 minutes,

after which the sample is transferred to the drying oven and dried at  $105^{\circ}$  to constant weight. A vacuum oven can be used, with advantage, for the final drying.

If the moisture residue is to be used for another estimation, such as nitrogen, it is convenient to use thin lead or tin-foil dishes or Hofmeister glass dishes which can be cut or broken, and placed in the apparatus used with the samples.

**Ash.** The ash in meat extracts may be estimated by charring a convenient quantity (2–10 grm.), extracting the char with hot water, ashing the char, and adding the extract, which is then evaporated to dryness, dried, and weighed. (See *General Methods for the Analysis of Meat*, p. 277.)

**Chlorine and Sodium Chloride.**—Chlorine may be determined by Volhard's method or gravimetrically by precipitation as silver chloride, working on a solution of the ash.

For factory control work, approximate estimations are made by charring a convenient quantity in a porcelain dish, grinding the char with a small agate pestle, and transferring the ground material by means of hot water to a 250 c.c. graduated flask. After making up to volume, the chlorine in an aliquot part is titrated with silver nitrate, neutral potassium chromate being used as indicator. The result is usually calculated to sodium chloride.

When the ash is to be used for the sodium chloride estimation care should be taken to keep the ignition temperature as low as possible in order to avoid volatilisation of sodium chloride.

**Total Phosphorus.**—The method described on p. 289 may be used in the case of meat extracts, the solution being made in nitrohydrochloric acid and the phosphorus weighed as magnesium pyrophosphate.

**Fat.** The fat content of meat extracts is usually very low, but it is difficult to extract. Petroleum spirit is the best solvent to use, as this extracts the fat only, whereas ethyl ether may dissolve other substances as well. If ethyl ether is used for the extraction, it should be anhydrous and alcohol-free. The extraction is carried out as for meat (see p. 284). Tin-foil or a glass dish can be used in which to dry the sample, and the drying should be done according to one of the methods given above, after which the sample is ground with sand and transferred to the extraction shell. A second drying in the vacuum oven is advisable at this stage. The extrac-



tion proceeds in the Soxhlet apparatus for 16-24 hours, and the final drying of the fat should take place in the vacuum oven for accurate results. In all cases the fat should be tested for solubility in petroleum spirit.

**Total Nitrogen, Nitrate and Nitrite Nitrogen, Ammoniacal Nitrogen.**—These are determined as described above. Richardson prefers distilling the aqueous solution with barium carbonate for the determination of ammoniacal nitrogen.

No analysis of a meat extract or similar preparation can be regarded as affording reliable information as to the quality of the sample, which does not make some distinction between the different forms in which the nitrogen exists. Thus while the extractive matters and meat bases have a special value of their own, they are not nutritive. Albumin, albumose, and peptone, on the other hand, are true nutritive compounds, and are superior in value to gelatin.

**Proximate Analysis and Determination of the Nitrogenised Constituents of Meat Extracts, Etc.**—In the fullest possible analysis of a meat extract an attempt will be made to discriminate between, and estimate the amount of, nitrogen existing in the various forms of meat fibre and insoluble albumin, coagulable albumin, acid-albumin, albumoses, peptones, coagulable gelatin, gelatin-peptone, meat-bases, amino compounds, and ammonia. Such an analysis is necessarily tedious and rarely necessary, but some of the more important of the above estimations can be effected with reasonable ease and accuracy, and are not uncommonly required of the analyst.

In consequence of the uncertainty attaching to the composition of certain of the nitrogen-containing constituents of meat extracts, it is often convenient to state simply the amounts of nitrogen found to exist in the various forms, and this also in cases where it is preferred to state the actual amounts of the nitrogen-containing substances present. The corresponding amounts of nitrogen should always be given in addition.

**Unaltered Proteins and Meat Fibre.**—Bovril and certain allied preparations contain finely powdered meat fibre. This may be detected by treating the meat extract with cold water, and examining the insoluble portion under the microscope. If meat fibre be found, 5 grm. of a dry preparation, 8 to 10 grm. of an extract, or 20 to 25

gram. of a fluid preparation should be treated with cold water, the insoluble matter collected on a filter, washed with cold water, dried at  $100^{\circ}$ , and weighed. The weight obtained represents the meat fibre and insoluble matter of the preparation. An alternative, and in some respects preferable, plan is to determine the nitrogen in the moist residue. Proceed as under insoluble nitrogen (p. 496). The nitrogen found, multiplied by 6.25, will give the insoluble meat-proteins, as distinguished from the crude meat fibre, etc., obtained by weighing the insoluble matter. If there is a large amount of insoluble matter, a larger quantity can be weighed out, made up to volume in a graduated flask, and the nitrogen estimated in an aliquot part after filtering through a folded filter and removing nitrates if present. The percentage of soluble organic nitrogen deducted from the percentage of total nitrogen gives the percentage of nitrogen in insoluble proteins.

*Coagulable albumin* can be estimated in the filtrate from the insoluble matter as shown above (p. 496). The filter paper used for the separation of these albuminous precipitates must be as free as possible from nitrogen, or a correction must be made for the amount present. Stutzer recommends the filters of Schleicher and Schüll. Only insignificant amounts of albumin are usually present in meat extracts, but in certain preparations which have received an addition of scale-albumin the amount may be considerable.

*Syntonin*.—An aliquot portion of the liquid filtered from the coagulable albumin should be further acidified with acetic acid and tested with potassium ferrocyanide. If any precipitate is formed, the liquid should be heated, and if re-resolution does not ensue, the presence of acid-albumin is certain. If found, the remainder of the liquid should be rendered exactly neutral to litmus, the precipitate filtered off, and the contained nitrogen estimated. Syntonin is stated by Denaeyer to be present in considerable proportion in "Kemmerich's meat peptone," while "Somatose" consists largely of alkali-albumin, which will be determined as syntonin by the above process.

*Albumoses (and Gelatin)*.—The filtrate from the precipitate of syntonin, or, in the absence of syntonin, the liquid filtered from the coagulable albumin, is treated as shown above (p. 496). The precipitate produced contains all the albumose of the extract, together with any gelatin which may be present and any coagulable or insolu-

ble proteins not previously removed. Peptones, meat bases, amino-compounds, and ammoniacal salts are not precipitated.

*Peptones (and Meat-bases).*—The most satisfactory method of estimating peptones is the tannin-salt method of Bigelow and Cook (*J. Amer. Chem. Soc.*, 1906, **28**, 1497). Although it leaves much to be desired, it offers the best method of separation at the present time, and it will be still better when a nitrogen-free tannic acid is obtainable.

One grm. of meat powders, 2 grm. of preparations of pasty consistency, and from 10 to 20 c.c. of liquid or semi-liquid extracts, should be employed. Solid and pasty preparations are dissolved in a little cold water in a 100 c.c. graduated flask, keeping the volume within 20 c.c. Proceed as for amino acid and extractive nitrogen in cold water extracts (p. 207). This method gives the meat base and the ammonium nitrogen directly. For calculating the meat bases, the factor 3.12 is used. The peptones are calculated by subtracting the sum of the various other forms of nitrogen from the total nitrogen.

*Creatine and Creatinine.*—For directions for these determinations see pages 272, 299 and 497.

Grindley and Woods (*J. Biol. Chem.*, 1907, **2**, 313) have published the following figures for creatine and creatinine in meat extracts:

CREATININE IN COMMERCIAL BEEF EXTRACTS

No. of sample	Weight of sample grm.	Reading of colorimeter mm.	Creatinine	
			Weight, mg.	%
1	0.3008	7.8	10.38	3.45
2	0.2545	8.6	9.42	3.70
3	0.5800	8.1	10.00	1.72
4	0.7221	8.0	10.13	1.40
5	0.1072	7.8	10.38	5.27
6	0.7234	8.2	9.88	1.37
7	0.8206	8.2	9.88	1.20
8	0.5458	7.7	10.52	1.93
9	1.1301	8.6	9.42	0.83
10	0.4720	8.1	10.00	2.12
11	0.6786	8.5	9.47	1.39
12	0.7540	7.5	10.08	1.30
13	0.2774	7.3	11.10	4.00

## CREATINE IN COMMERCIAL BEEF EXTRACTS

No. of sample	Weight of sample, grm.	Reading of colorimeter mm.	Weight of original creatinine, plus creatinine, due to creatine, mg.	Weight of original creatinine, mg.	Weight of creatinine due to creatine (A) mg.	Weight of creatine $A \times 1.16$ mg.	Creatine %
2	0.1833	7.9	10.25	6.78	3.47	4.03	2.18
3	0.2636	7.7	10.52	4.55	5.97	6.93	2.63
4	0.1850	7.9	10.25	2.60	7.65	8.74	4.79
5	0.1512	8.4	9.64	7.06	1.68	1.95	1.29
6	0.1841	8.6	9.42	2.38	7.04	8.17	4.35
7	0.1641	8.0	10.13	1.98	8.15	9.45	1.20
8	0.2593	8.0	10.13	5.00	5.13	5.95	2.29
9	0.7088	8.8	0.20	5.86	3.34	3.87	0.55
11	0.3016	5.0	10.20	4.19	12.01	13.93	4.62
12	0.2262	6.85	11.82	2.94	8.88	10.30	4.55
13	0.2080	7.00	11.57	8.32	3.25	3.77	1.81

## COMBINED CREATININE AND CREATINE IN COMMERCIAL BEEF EXTRACTS

No. of sample	Creatinine %	Creatine %	Sum of creatinine and creatine %
2	3.70	2.18	5.88
3	1.72	2.63	4.35
4	1.40	4.79	6.19
5	5.27	1.29	6.56
6	1.37	4.35	5.72
7	1.20	1.20	2.40
8	1.93	2.29	4.22
9	0.83	0.55	1.38
11	1.30	4.62	6.01
12	1.30	4.55	5.85
13	4.00	1.81	5.81

*Xanthine Bases.*—Bigelow and Cook (see tables, pp. 479-480) have also estimated the xanthine bases in their work on meat extracts. Their remarks under this head and their method follow:

"In addition to creatine and creatinine, a true meat extract or meat juice should contain small amounts of xanthine bases, including xanthine, hypoxanthine, guanine, and adenine. These substances are derived from the nuclei of the cells, and, consequently, in an extract that is prepared from fresh, unaltered beef, a certain

amount of these substances should be obtained together with the salt and other extractive matter. The estimation of the xanthine bases is, therefore, of value in estimating the origin of an alleged extract of meat.

"The xanthine base figures in the tables show a variety of results, which is explained by the fact that in the preparation of the extract under certain conditions of heat and pressure some of these substances are destroyed. The following modification of Schittenhelm's method was employed for their estimation:

"Use an amount of the standard solution equivalent to 5 gm. of the original extract. Place in a large evaporating dish and add 500 c.c. of 1% sulphuric acid. Evaporate to 100 c.c. within 4 to 5 hours. Cool and neutralize with sodium hydroxide. Add 10 c.c. of 15% sodium disulphate, and 15 c.c. of 20% copper sulphate; allow this to stand overnight, filter, and wash. The precipitate suspended in water is treated with sodium sulphide and warmed on the steam-bath. Add acetic acid to acidify and filter hot. To the filtrate add 10 c.c. of 10% hydrochloric acid and evaporate to a volume of about 10 c.c. Filter, make ammoniacal, and add ammoniacal silver nitrate of 3% strength. After standing several hours the solution is filtered and washed with distilled water until no longer alkaline. The nitrogen in the precipitate is that of the xanthine bases."

**Other Methods for Meat Bases.**—Various methods for the separation and estimation of meat bases have been proposed, but have not come into general use because of their length and complexity. One of the older methods, in brief, consists in first boiling the solution and filtering off the albumin, concentrating the filtrate to small bulk, and adding lead acetate to remove sulphates, phosphates, and chlorides. The filtrate is freed from lead by adding hydrogen sulphide and filtering, and the latter removed by concentrating to a syrup. After long cooling, creatine crystallises out. The crystals are filtered off, washed with 88% alcohol and weighed. After removing alcohol from the filtrate and digesting for 3 hours with dilute sulphuric acid (1:3) the xanthine bases are precipitated by the copper bisulphite method of K. Micko (*Z. Nahr. Genussm.*, 1902, **5**, 193; 1903, **6**, 781). See also König (*Nahrungs und Genussmittel*, **3** (i), 314-17). From the filtrate from the xanthine bases creatinine is obtained as the zinc chloride compound.

*Kutscher's Method* (*Z. Nahr. Genussm.*, 1905, **10**, 528; 1906, **11**, 582).—Kutscher has developed a method for the separation and estimation of the meat bases and by means of it has discovered several new bases in meat extract. Some of the bases he has named Ignotine, Carnomuscarine, Neosine, Novaine, and Oblitine, respectively. Kutscher worked on about 450 grm. of Liebig's meat extract, and this he treated with 500–600 grm. of tannin in solution. Purification of the filtrate from the tannin precipitate was effected by means of barium hydroxide, filtration, dilute sulphuric acid, and lead oxide and filtration. The filtrate was then rapidly concentrated to a syrup. Lead compounds separated at first, then creatine crystals. Upon standing for 24 to 48 hours more crystals separated, consisting chiefly of creatine, and creatinine. The crystals were filtered off quickly and washed with a small quantity of ice-cold water. The somewhat alkaline filtrate (from the lead oxide) was acidified with sulphuric acid, filtered from the lead sulphate, and the new filtrate precipitated with 20% silver nitrate. The precipitate consisted chiefly of silver chloride, alloxan bases, and others, and was filtered off after 24 hours. The filtrate was again treated with 20% silver nitrate until, upon testing, no longer a white but a brown precipitate was formed in saturated baryta water. Baryta water was then added in slight excess. The silver precipitate was treated first with sulphuric acid then with hydrogen sulphide, and the filtrate concentrated to a syrup. From this syrupy solution the new bases were separated. For complete directions the analyst should consult Kutscher's original papers.

*Ammonium Salts.*—There is no satisfactory method for the estimation of ammoniacal nitrogen in meat extracts. An approximation can be obtained by distillation with magnesium oxide or barium carbonate, but the results are too high, and there is no definite limit to the evolution of ammonia. The air aspiration method (p. 327) may be used, but it is extremely slow, and the results are of doubtful accuracy, unless the method is most carefully carried out and checked.

*Gelatin* is present in notable quantity in some meat extracts, and its estimation presents considerable difficulty. Gelatin is precipitated more or less completely by most of the reagents for proteins, including tannin, phosphotungstic acid, bromine water, and ammonium and zinc sulphates. Unchanged gelatin is said not to be

precipitated by Stutzer's copper hydroxide reagent, but the commercial article is largely thrown down. The same is the case when a solution of commercial gelatin (Nelson's) is treated with mercuric chloride or with potassium mercuric iodide, although these reagents are stated by Denaeyer not to precipitate gelatin; in fact, the first reagent is employed by him to separate gelatin from peptone, and the second to differentiate gelatin from albumose.

Unchanged gelatin is precipitated by alcohol of very moderate strength (50 to 60%), but the non-jellifiable modification produced by the prolonged action of hot water or of weak acids on gelatin is only precipitated by very strong alcohol (95%). This modified gelatin, often called *gelatin-peptone* or *gelatone*, has been but imperfectly examined, but differs materially from colloidal gelatin in its chemical reactions.

Various experiments were conducted in Allen's laboratory by A. B. Searle with a view of finding a reliable chemical method of separating gelatin from proteins. So far, none of the reagents generally credited with effecting a separation of gelatin from albumoses have been found to behave in accordance with published statements, and no method of distinguishing sharply between gelatin and gelatin-peptone has been devised up to the time of writing.

On the whole, the gelatin of meat extracts is best determined by the following modification by Stutzer of a method devised by Denaeyer, but the process can not be regarded as wholly satisfactory:

The tin-foil capsule containing the dry residue resulting from the estimation of the total solids of the sample (page 500), together with the sand, is cut into small strips and placed in a beaker.

The following is Stutzer's final method of operating (*Z. anal. Chem.*, 1895, **34**, 568): The beaker (marked *a*) together with four others (marked *b*, *c*, *d*, *e*) and a flask containing a mixture of 100 c.c. of alcohol, 300 gm. of ice, and cold water to 1 kgrm., are then immersed in a bath containing crushed ice. About 100 c.c. of the mixture in the flask, the temperature of which must not exceed 5°, is poured on the sand, stirred with a glass rod for 2 minutes, and decanted into beaker *b*, a piece of ice being added to keep down the temperature. The extraction in the beaker is repeated with a second quantity of alcoholised water which is poured into *c*, and the treatment repeated until the last washing is colourless, a fragment

of ice being added to each quantity of extraction liquid as soon as it is poured off. Three funnels, of about 7 cm. diameter, are then arranged with filter-beds of long-fibred asbestos supported by perforated porcelain plates about 4 cm. in diameter, and connected with a pump by which gentle and gradually-increasing suction can be applied. The contents of beaker *a* are filtered into the first, *b* is poured into the second, and *c*, *d*, and *e* into the third. The filters, as well as that through which the absolute alcohol extract has been filtered, are then thoroughly washed with the ice-cold alcoholised water, transferred to a porcelain basin, and repeatedly extracted by boiling with water. The aqueous extract is filtered, concentrated, and treated by Kjeldahl's process.

Stutzer finds that, when the process is conducted exactly in the manner prescribed above, from 95 to 98% of the total gelatin present is obtained, and that the small quantities of gelatin-peptone present in meat extracts are precipitated by alcohol, together with the gelatin proper, and may be suitably estimated therewith. The nitrogen found, multiplied by 5.55, gives the gelatin of the sample.

**Qualitative Test for Yeast Extracts.**—A product made from waste yeast and used as a substitute or an adulterant of meat extract came on the market some years ago, especially in Germany. Bigelow and Cook report as follows (*U. S. Dept. of Agric. Bur. of Chem. Bull.*, 114). See also F. C. Cook, A Comparison of Beef and Yeast Extracts of Known Origin. *U. S. Dept. Agric. Bur. Chem. Circular* 62, 1910) on these preparations:

"Searle (*Pharm. J.*, 1903, 71, 516 and 704; 1904, 72, 86) suggests as a method for detecting yeast products added to meat preparations, that a solution of the extract be boiled for 1 or 2 minutes with a modified Fehling's solution. In the presence of yeast extract a bluish-white precipitate is obtained. Arnold and Mentzel (*Pharm. Ztg.*, 1904, 49, 176) claim that a slight bluish-white precipitate is given even by pure meat extracts, but by experience an analyst learns to detect by this method the presence of about 20% of yeast extract in meat preparations. Micko (*Z. Nahr. Genussm.*, 1902, 5, 193; 1903, 6, 781) suggests the estimation of creatine and xanthine substances as a means of determining the nature of the extract. Wintgen (*Arch. Pharm.*, 1904, 242, 537) states that the filtrate from the zinc sulphate precipitate obtained in the estimation of albumoses



is entirely clear in the case of meat extracts, but somewhat turbid with yeast extracts. This he finds to be true even when the best Schleicher & Schüll filter paper is employed. By this method the authors could detect from 20 to 30% of added yeast extract.

"E. Baur and H. Barschall (*Arb. kaiserl. Gesundheitsamte*, 1906, 24, 562) have applied the colorimetric test, as outlined by Folin, for creatinine to meat and yeast extracts. They find no creatine or creatinine in yeast extracts, and base a distinction between the two on this test. Salkowski (*Ber.*, 1894, 27, 499) has studied the various carbohydrates of yeast and gives several tests for yeast gum.

"The most reliable test is unquestionably the estimation of creatine. A yeast extract contains no creatine, and in a typical meat extract there is found from 10 to 20% of the total nitrogen in the form of creatine and creatinine. The distribution of the various xanthine bases also is different in the two kinds of extracts; in meat extract, according to Micko (*loc. cit.*), xanthine and hypoxanthine predominate, whilst in yeast extracts adenine and guanine predominate.

"A test for yeast extracts consisting in boiling the samples for 1 or 2 minutes with an unmodified Fehling's solution was tried. Four samples were tested with the following results:

	Colour of precipitate
A. Meat extract	Very deep violet colour
B. Yeast extract	Very deep green colour
C. 50% yeast and 50% meat extract	Intermediate colour
D. 25% yeast and 75% meat extract	Violet colour, not as strong as A

This test is of value as a qualitative and a confirmatory test for yeast extracts in the presence of meat extracts.

"The method of Searle for the detection of yeast extract by the use of a modified Fehling's solution is as follows:

"Prepare a modified Fehling's solution by dissolving 12.96 gm. of copper sulphate and 16.20 gm. of neutral tartrate of sodium in 113.4 c.c. of water. Add to this 16.20 gm. of sodium hydroxide dissolved in 113.4 c.c. of water. Dissolve 0.6481 gm. of the sample to be examined in 42.5 c.c. of water, add to this  $\frac{1}{2}$  volume of the above solution, and boil for 1 or 2 minutes. With genuine meat extract no precipitate is given. When yeast extract is present a curdy, bluish-white precipitate is formed.

"This method was tested on a sample of meat extract, a yeast extract, a 50% solution of yeast and meat extract, and a solution containing 20% of yeast and 80% of meat extract. In the case of the meat extract a very fine precipitate was obtained. In the three cases where yeast extract was present a flocky, bluish-white precipitate was formed. It is evident from these results that the presence of 20% of yeast extract in meat mixtures may be detected by this method.

"Another test is described by Wintgen (*Arch. Pharm.*, 1904, **242**, 537), who claims that the zinc sulphate filtrate in the case of meat extracts is clear, but with yeast extracts it is turbid. This was found to be the case, as the following results show:

	Zinc sulphate filtrate
A. Meat extract	Clear
B. Yeast extract	Cloudy
C. 50% yeast and 50% meat extract	Cloudy
D. 25% yeast and 75% meat extract	Cloudy

The solutions of these extracts, or mixtures were saturated with chemically pure zinc sulphate after adding 2 drops of strong sulphuric acid. The solutions stood overnight and the filtrates were examined in the morning. The only clear filtrate obtained was that from meat extract alone.

The most important test for determining the nature of an extract, whether meat or yeast, is the determination of creatine and creatinine. This test, which has been used in the Bureau of Chemistry for 2 or 3 years and found to be of great value, was perhaps first applied by Micko (*loc. cit.*). As before stated, yeast extracts contain no creatine or creatinine, whilst in meat extracts these two bodies are present in considerable amounts.

"Some experiments on meat extract, yeast extract, and mixtures of the two were tried with satisfactory results, using the Folin (*Z. physiol. Chem.*, 1904, **41**, 223) colorimetric method. In estimating the creatinine by this method in the presence of yeast extract, slightly higher results are obtained than when yeast extract is not present. When the creatine and creatinine are determined together (after dehydrolysis) in a sample of meat extract the presence of yeast extract does not seem to affect the results. In the case of the yeast extract no creatine or creatinine was found.

## CREATININE IN MEAT AND YEAST EXTRACTS—BIGELOW AND COOK

No.	Description of sample	Weight of sample gram.	Creatinine		Creatinine calculated to meat extract used %	Increase of creatinine due to presence of yeast extract %
			Weight mg.	%		
1	Meat extract.....	{ 0.2660 0.2663	8.804 8.804	3.39 3.31	3.39 3.31	0 .....
	Average.....			3.35	3.35	
2	Yeast extract.....	0.4800	0	0	0	0
3	Mixture—50% meat and 50% yeast extract.....	{ 0.4154 0.3756	7.788 7.013	1.87 1.87	3.75 3.75	..... 0.40
	Average.....			1.87	3.75	.....
4	Mixture—75% meat and 25% yeast extract.....	{ 0.2638 0.3030	8.437 7.941	3.19 2.62	4.25 3.40	..... 0.52
	Average.....			2.91	3.87	.....

## TOTAL CREATININE (INCLUDING CREATINE CONVERTED INTO CREATININE)

## In Meat and Yeast Extracts

No.	Description of sample	Weight of sample gram.	Creatinine		Creatinine calculated to meat extract used %	Increase of creatinine due to presence of yeast extract %	Creatine calcu- lated as creatinine (by dif- ference) %
			Weight mg.	%			
1	Meat extract.....	{ 0.2210 0.2144	11.571 9.870	5.24 4.60	5.24 4.60	0	4.92-3.35 = 1.57
	Average.....			4.92	4.92		
2	Yeast extract.....	5250	0	0	0	0	0
3	Mixture—50% meat and 50% yeast extract.....	{ .4378 .4020	11.571 9.691	2.64 2.41	5.29 4.82	..... 0.13	5.05-3.75 = 1.30
	Average.....			2.53	5.05	.....	.....
4	Mixture—75% meat and 25% yeast extract.....	{ .3554 .3255	15.577 10.385	4.38 3.19	5.85 4.25	..... 0.13	5.05-3.87 = 1.18
	Average.....			3.79	5.05	.....	.....

**COMPARATIVE ANALYSIS OF BEEF AND YEAST EXTRACTS—COOK**  
On Basis of Original Sample

Determinations	Yeast extract		Beef extract			
	A.P. 724 %	A.P. 725 %	A.P. 794 %	A.P. 795 %	A.P. 796 %	A.P. 797 %
Solids .....	73.73	72.34	84.78	86.23	79.73	86.66
Total ash .....	22.18	19.48	19.57	16.23	14.63	14.49
Chlorine of ash as sodium chloride ...	2.38	1.72	1.67	2.07	2.05	1.83
Phosphoric acid:						
Of ash .....	2.71	2.76	2.30	2.63	1.96	2.22
Total .....	2.73	2.78	2.50	3.00	2.04	2.27
Organic .....	0.24	0.29	0.25	0.27	0.20	0.25
Ether extract .....	0.83	1.01	6.33	7.85	6.34	6.95
Nitrogen:						
Total .....	5.40	5.31	9.43	9.43	8.63	9.13
Proteose and peptide <sup>1</sup> .....	2.15	2.18	3.40	3.04	1.89	2.39
Amino .....	3.25	3.13	6.03	6.39	6.74	6.74
Purine base .....	0.58	0.64	...	0.65	0.77	0.64
Creatine .....	0	0	4.00	2.90	1.51	1.95
Creatinine .....	0	0	2.22	2.60	2.35	2.62
Acidity <sup>2</sup> .....	9.86	10.27	11.23	10.54	10.14	12.33

<sup>1</sup> This figure includes about 25 % of the total creatine and creatinine nitrogen.

<sup>2</sup> Expressed as c.c. of N/10 sodium hydroxide per gram.

**COMPARATIVE ANALYSIS OF BEEF AND YEAST EXTRACTS—COOK**  
(Continued)

Calculated to a Moisture-free and Fat-free Basis

Determinations	Yeast extract		Beef extract			
	A.P. 724 %	A.P. 725 %	A.P. 794 %	A.P. 795 %	A.P. 796 %	A.P. 797 %
Total ash .....	30.42	27.32	24.94	20.71	19.03	18.18
Chlorine of ash as sodium chloride .....	3.27	2.41	2.13	2.64	2.79	2.30
Phosphoric acid:						
Of ash .....	3.72	3.87	2.93	3.36	2.64	2.79
Total .....	3.74	3.89	3.19	3.83	2.78	2.85
Organic .....	0.33	0.41	0.32	0.35	0.27	0.31
Nitrogen:						
Total .....	7.41	7.45	12.02	12.03	11.76	11.46
Proteose and peptide <sup>1</sup> .....	2.95	3.06	4.33	3.88	2.58	3.00
Amino .....	4.46	4.39	7.70	8.15	9.10	8.46
Purine base .....	0.80	0.90	...	0.83	1.05	0.80
Creatine .....	0	0	5.10	3.70	2.06	2.44
Creatinine .....	0	0	2.83	3.32	3.20	3.28
Acidity <sup>2</sup> .....	13.52	14.40	14.31	13.45	13.82	15.47

<sup>1</sup> This figure includes about 25 % of the total creatine and creatinine nitrogen.

<sup>2</sup> Expressed as c.c. of N/10 sodium hydroxide per gram.

Cook reports the preceding figures showing the comparative composition of meat and yeast extracts of known origin (*U. S. Dept. Agric. Bur. Chem., Circular 62, 1910*).

**Beef Extract Cubes.**—A number of preparations are now on the market, known by such names as "Oxo," "Steero," "Beef Cubes," "Bouillon Cubes," etc., which consist mainly of nearly dry meat extract, salt, dried ground beef, and such flavouring matters as finely ground celery and tomato seed, tomato pulp, etc. These preparations are sold in the form of small cubes, rather less than  $\frac{1}{2}$  inch on an edge, which are separately wrapped in metal foil, and packed to the number of 1 or 2 dozen in small tinned boxes. It is stated that 1 cube will make a cup of broth by the simple addition of hot water.

The methods of analysis for meat extracts apply to these products.

**Assay of Meat Extracts by Alcohol Precipitation.**—A simple means of roughly differentiating between the soluble forms of nitrogen-containing matters in meat extracts has been in use for many years in the laboratories of manufacturers of such preparations. It consists in treating the sample with alcohol of such strength as to precipitate as much as possible of the protein and gelatinoid constituents of the extract, and as little as possible of the meat extractives and salts. For this purpose, O. Hehner recommends (*Analyst*, 1885, **10**, 221) that 2 grm. of the sample should be dissolved in 25 c.c. of water, and 50 c.c. of strong methylated spirit added to the solution. The precipitate is allowed to settle overnight, and the clear liquid then decanted as completely as possible. The (unwashed) precipitate is dissolved in a little hot water, the solution evaporated in a weighed basin, and the residue dried at 100° and weighed.

O. Hehner (*Analyst*, 1885, **10**, 221) gives the results shown on p. 515 of analyses of some well-known preparations which he examined by the above method. The results yielded by two samples of "Essence of beef" of South African manufacture, analysed in Allen's laboratory by the same process, are added for the purpose of comparison on p. 515.

J. Bruylants (*J. Pharm. Chim.*, 1897, **5**, 515) has described a method of analysing meat extracts, based on fractional precipitation by alcohol of different strengths. K. Micko has found Bruylants' method to work well (*Z. allgem. Österreich. Apotheker-Vereins*, **30**, 1). Thus, gelatin is thrown down by alcohol of 40%, albumoses

Description	Water %	Total solids %	Alcohol precipitate %	Ash %	Phosphoric acid %	Nitrogen %	Authority
Liebig's extract	18.70	81.30	3.16	23.38	6.07	7.94	O. Hehner
Nelson's gelatin			93.19	3.25 <sup>1</sup>	None		O. Hehner
Concentrated beef tea.							
English	36.96	63.04	27.40	4.36	1.16	8.25	O. Hehner
English	31.00	69.00	30.30	4.13	1.00	8.36	O. Hehner
English	41.93	58.07	25.50	4.92	1.10	7.52	O. Hehner
Russian	24.56	75.44	35.40	6.72 <sup>2</sup>	0.95	9.89	O. Hehner
X	54.31	45.69	32.30	7.57	2.11	6.79	O. Hehner
Commercial essence of beef:							
English	89.25	10.75	3.07	1.17	0.34	1.36	O. Hehner
English	89.61	10.39	3.71	1.90	0.26	1.36	O. Hehner
English	92.32	7.68	1.99	1.30	0.38	0.79	O. Hehner
South African	90.50	9.50	2.98	1.74	0.50	1.22	A. H. Allen
South African	87.55	12.45	2.88	2.36	0.17	1.41	A. H. Allen

<sup>1</sup> This ash was insoluble in water, and consisted chiefly of calcium carbonate.

<sup>2</sup> This ash was only partly soluble in water, and was almost entirely composed of calcium carbonate. The ashes of the other samples, with the exception of Nelson's gelatin, were completely soluble in water, and practically devoid of lime.

The phosphoric acid was determined in the samples by precipitation with molybdate solution, re-solution of the precipitate in ammonia, evaporation of the solution at 100°, and calculation of the residue to P<sub>2</sub>O<sub>5</sub>.

by 80%, and peptones by 93 to 94% alcohol. The following results were obtained by the analysis of typical preparations:

	Liebig's extract %	Solid Bovril %	Bovril for invalids %	Liquid Bovril %
Water	16.75	19.20	2.35	43.25
Sodium chloride	2.95	4.50	4.60	9.75
Other mineral salts	18.24	16.20	17.05	6.25
Insoluble in water (meat fibre)		1	7.10	8.19
Organic matters	62.06	60.10	54.50	32.06
Total nitrogen	9.30	8.85	9.12	4.85
Nitrogen in part insoluble in water		1	1.09	1.19
Nitrogen, ammoniacal, urea, acid, etc.	0.60	0.50	0.45	0.30
Nitrogen, from lead precipitate non-protein matters	0.65	0.57	0.45	0.27
Nitrogen, non-protein from 80% alcohol	0.15	0.20	0.18	0.95
Nitrogen, soluble in strong alcohol	3.60	3.20	3.40	1.05
Nitrogen, from gelatin	0.19	0.25	0.12	0.05
Nitrogen, from albumoses	0.80	0.95	0.75	0.45
Nitrogen, from peptones	2.94	2.58	2.70	1.33
Total soluble proteins	24.56	23.62	22.40	11.43
Insoluble albumin (meat fibrin)		1	6.81	7.43

<sup>1</sup> The meat fibre in this sample does not appear to have been separately determined.

All methods of examining meat extracts based on precipitation of various proteins by alcohol of certain strengths, though useful in practice, are open to objection as deficient in accuracy. The method has been subjected to criticism by König and Bömer (Z.

*anal. Chem.*, 1895, **5**, 548; abst. *Analyst*, 1896, **12**, 17), who point out that in meat extracts prepared at low temperatures, and which are only concentrated to the required consistency after filtration, the amount of gelatin present must be excessively small.

**Assay of Meat Extract by Bromine Precipitation.**—The fact that aqueous solutions of the proteins and of gelatin are precipitated by chlorine has been utilised by Rideal and Stewart (*Analyst*, 1897, **22**, 228) as the basis of a process of assaying meat extracts, etc. As employed by these chemists, the method consists in passing a current of chlorine gas through the solution to be tested, filtering off and washing the resulting precipitate, and weighing it after drying at a temperature not exceeding 70° or preferably *in vacuo* over sulphuric acid. The precipitate is stated to be remarkably stable at ordinary temperatures, but to be readily decomposed on heating, becoming nearly black and rotting the filter paper.

In order to avoid the inconvenience attaching to the use of chlorine gas, and the drying and weighing of an unstable precipitate, Allen devised a modified process (*Analyst*, 1897, **22**, 258), which is rapid, easily worked, and gives concordant results. In this method, bromine water is employed as the precipitant, in place of chlorine. The precipitate is filtered off on asbestos, treated with strong sulphuric acid while still moist, and the contained nitrogen determined by the Kjeldahl-Gunning process (see page 286). The following are the details of the operation:

A quantity of the solution containing about 1 grm. of the albuminoid matter is diluted with cold water to a volume of about 100 c.c., and treated in a conical beaker with sufficient hydrochloric acid to render the liquid distinctly acid to litmus. Bromine water is then added in considerable excess, and the liquid stirred vigorously for some time. The yellowish precipitate which separates is at first flocculent, but becomes more viscous on stirring, and finally adheres in great part to the sides of the beaker. When this occurs the liquid is allowed to stand at rest for about  $\frac{1}{2}$  hour, or until the precipitate has settled. It is then decanted through an asbestos filter. The filter is made by placing a plug of glass-wool in a cylindrical funnel (constructed of a vertical glass tube drawn out at the lower end), and covering it with a pad of pulped asbestos. If the filter is properly constructed, no water-pump will be required, and a perfectly clear filtrate will be obtained.

The precipitate adhering to the sides of the beaker is washed several times with cold distilled water, the washings being poured through the filter. Occasionally, when the greater part of the bromine has been washed out of the precipitate, the liquid does not filter clear. It is, therefore, advisable to keep the washings separate from the filtrate, and, if necessary, to add bromine or sodium sulphate to the wash-water.

The contents of the filter-tube (including the asbestos, and, if necessary, the glass wool) are returned to the beaker used for the precipitation, 20 c.c. of strong sulphuric acid added, and the beaker covered with a watch-glass and heated over wire gauze. The substance chars and bromine vapour is evolved. When frothing has ceased, about 10 grm. of powdered potassium sulphate should be added, and the liquid boiled vigorously until colourless. It is then allowed to cool, diluted with water, an excess of sodium hydroxide added, the ammonia distilled off, and the distillate titrated with standard acid. From the nitrogen found the amount of protein or gelatinoid body present is deduced by a suitable factor.

As the results of experiments by the foregoing process, it was found (Allen and Searle, *Analyst*, 1897, **22**, 259) that practically the whole of the nitrogen of gelatin, gelatin-peptone, egg-albumin, syntonin, and of the mixed products of the acid-pepsin digestion of egg-albumin was thrown down in the precipitate produced by bromine. The following is a tabular statement of the chief results obtained:

Substance	Nitrogen %		Nitrogen multiplied by factor		Factor employed
	Total in original substance	Precipitated by bromine	Total in original substance	Precipitated by bromine	
Commercial gelatin.....	14.10	14.00	76.42	76.14	5.42
Gelatin-peptone.....	14.10	13.90	76.42	75.44	
Commercial scale-albumin.....	8.80	8.72	55.8	55.2	
Syntonin from scale-albumin.....	9.86	9.76	62.41	61.78	6.33
Digested scale-albumin..	8.89	8.81	56.3	55.8	
Fresh white of egg.....	1.89	1.88	11.96	11.90	
Syntonin from white of egg.....	1.89	1.89	11.96	11.96	
Peptone from white of egg	0.70	0.69	4.43	4.37	
Beef extractives.....	0.33	0.004	2.11	0.03	



In the case of syntonin, fairly good results were obtained whether the acid used for conversion of the egg-albumin was left unneutralised or was exactly saturated by sodium hydroxide; but if the solution was made alkaline and bromine then added, the protein could not be completely precipitated by subsequent free acidification of the liquid. The mixed peptones formed by the acid-pepsin digestion of the white of hard-boiled eggs were also completely precipitated by bromine.

A sample of commercial scale-albumin was converted into acid-albumin (syntonin) by heating it in 1% solution with hydrochloric acid for 6 hours. The nitrogen was then estimated directly in the resultant liquid and in the bromine precipitate produced in different ways as shown below. The figures are calculated to 100 parts of the original albumin, which was found to contain 9.88% of nitrogen and to yield 8.32% of ash on ignition. Hence the sample was far from pure.

	Nitrogen %
A. By direct Gunning-Kjeldahl process on syntonin solution . . .	9.86
B. By precipitate from unneutralised syntonin solution . . . . .	9.76
C. By precipitate from nearly neutralised syntonin solution . . .	9.69
D. By precipitate from syntonin solution, rendered strongly alkaline and then re-acidified . . . . .	9.60
E. Precipitate from syntonin solution, made strongly alkaline by soda, bromine added, and the liquid acidified after $1\frac{1}{2}$ hour . .	6.69
F. Precipitate from syntonin solution, made strongly alkaline by soda, bromine added, and the liquid acidified after 24 hours . .	3.52

On the other hand, bromine produced no precipitate in acidified solutions of creatine, creatinine, asparagine, or aspartic acid. A meat extract, prepared by soaking raw beef in 10 parts of cold water, straining, boiling, and filtering from the coagulated albumin, gave only a trifling precipitate on addition of bromine water. In the liquid concentrated to one-tenth a larger precipitate was obtained, the greater part of which dissolved on diluting the liquid with an equal volume of water, and almost the whole on addition of a few drops of hydrochloric acid. On the other hand, the complete precipitation of albumin and gelatin by bromine seemed to be quite unaffected by dilution or the presence of free hydrochloric acid.

On applying the bromine method to commercial meat extracts the following results were obtained. The solutions were not previously filtered, and therefore, the figures include the nitrogen of any meat fibre present in the preparations:

	Nitrogen in precipitate by bromine %	Proteins = $N \times 6.3$ %
Liebig Company's Extract.....	1.41	8.88
Seasoned Bovril.....	1.94	12.22
Bovril for Invalids.....	2.64	16.63
Brand's Beef Bouillon.....	1.52	9.58
Vimbos.....	1.83	11.53

Another sample of Liebig Company's extract, analysed in Allen's laboratory by entirely different methods, gave 9.37% of total proteins.

König and Bömer have shown that the protein nitrogen in meat extracts has generally been much over-estimated. They found a total of 1.17% of protein nitrogen in Liebig Company's extract, which is equivalent to 7.41 of total proteins (mostly albumose).

In another experiment, 5 grm. of the Liebig Company's extract was dissolved in 100 c.c. of water, and the solution saturated with zinc sulphate. On adding bromine to the filtered liquid, a precipitate was produced which redissolved on diluting with water and adding hydrochloric acid. When 50 c.c. of the filtrate from the zinc sulphate precipitation was diluted with water to 250 c.c., and freely acidified with hydrochloric acid, no precipitate was produced on subsequently adding bromine. This result appears to negative the presence of considerable quantities of real peptones in Liebig's extract, and confirms the conclusion of König and Bömer on this point.

A parallel experiment with Bovril gave a precisely similar result.

Various other reagents for separating the nitrogenous substances of meat extracts have been proposed and used at various times, such as phosphotungstic acid, formaldehyde, chromic acid, potassium-mercuric iodide, etc. (See pp. 485, 514 and the second edition of this work, pp. 326-330.)

Most of these methods have been abandoned in favour of those detailed on previous pages.

**Non-nitrogenous Extractive Matters.**—These consist of lactic acid, succinic acid, glycogen, added glycerol, sugar, starch, etc.

Of these constituents of meat extracts very little is known quantitatively. *Lactic acid* and *lactates* probably predominate, but their actual amount does not appear to have been ascertained. *Glycogen*

is present in sensible quantity, and is determined by dissolving the sample in a little water and precipitating the solution with alcohol of 60%. The resulting precipitate is treated with a dilute solution of potassium hydroxide and the solution obtained is treated by Pflüger's method (p. 266).

Kemmerich states that meat extract is free from dextrin, sugar, and similar substances, and contains no substance which is converted into glucose by boiling with dilute sulphuric acid. Cured meat extracts from sweet pickle meats naturally contain *sugar*.

The *salts* of meat extracts have already been considered. They consist chiefly of *earthy phosphates* and *potassium chloride* and *acid phosphate*. Lactates and other organic salts of potassium are also present, and on ignition of the residue obtained by evaporating the extract are, of course, converted into carbonates. The acid potassium ortho-phosphate is also decomposed with formation of meta-phosphate ( $\text{KH}_2\text{PO}_4 = \text{KPO}_3 + \text{H}_2\text{O}$ ). These reactions necessarily affect the amount and composition of the ash, and should be borne in mind.

Taking the proportion of lactic acid in fresh meat at 0.06%, and assuming that 34 parts of meat are required for the production of 1 part of extract, the proportion of lactic acid in the latter would be 2.04%.

Cured meat extracts contain, besides the salts enumerated, sodium chloride, potassium nitrate, sodium nitrate or sodium nitrite or both. The last named salts may be estimated by the methods shown on p. 418 *et seq.*, after a qualitative test with diphenylamine in sulphuric acid.

**Extraneous Matters.**—In addition to *meat fibre*, the detection and estimation of which has already been described (p. 502), other foreign matters are present in certain commercial preparations classed broadly as meat extracts. In the table on p. 475 there are several instances of preparations containing *glycerin*, and one in which a *vegetable extract* was present. *Albumin* and *gelatin* are sometimes added as such. *Glucose* and *milk-sugar* are sometimes present.

*Alcohol* is an occasional constituent of "meat juices."

It is not practicable to draw a sharp distinction between meat extracts and the so-called "peptones" of commerce.

*Boric acid* was formerly added to meat extracts as a preservative, and, some years ago was found by Allen in notable quantity in a

widely-used preparation. A process proposed by C. Fresenius and Popp (abst. *Analyst*, 1897, **22**, 282) and applied by them to the examination of sausages, etc., may be employed for the estimation of boric acid in meat extracts. An amount of the extract corresponding to about 3 grm. of dry substance should be concentrated to a syrup, if necessary, and mixed in a mortar with from 40 to 80 grm. of recently ignited sodium sulphate. The mixture is heated in the water-oven for about an hour, and as soon as the mass is dry, some more sodium sulphate is added, and the whole reduced to a fine powder. This is digested with 100 c.c. of cold methyl alcohol for 12 hours, with frequent shaking, after which the alcohol is distilled off. As a rule the boric acid passes over completely in one distillation, but it is desirable to extract the residue a second time, using 50 c.c. of methyl alcohol. The distillate is made up to 150 c.c., and 50 c.c. treated with 75 c.c. of water and 25 c.c. of pure glycerol. The mixture is titrated with  $N/10$  solution of sodium hydroxide (free from carbonate), phenolphthalein being used as an indicator. A pale rose colour indicates the end of the titration. When it appears, some more glycerin should be added, and if the colour is not permanent the titration is continued till that point is attained. The volume of alkali used (in c.c.), multiplied by 0.0031, gives the boric acid,  $H_3BO_3$  (in grm.) in the volume of the distillate titrated. Borates will be dissolved out of the organic matters by the methyl alcohol, but will not pass over with the free boric acid. They may be determined in the usual manner in the methyl alcoholic extract, after evaporation, ignition, etc.

The method of estimating boric acid given under cured meats, pp. 426 and 440, may also be used for its estimation in meat extract.

**British Critique of Methods in Use.**—The methods adopted in the determination of nitrogenous constituents of extracts derived from albuminous substances, such as meat extracts and similar products, were discussed in a symposium reported in the journal of the Society of Public Analysts (*Analyst*, 1915, **40**, 310). A number of workers participated in a discussion of the methods in use. Chapman stated that the bromine method, when properly employed, precipitated gelatin, gelatin-peptone, syntonin, albumoses, and peptones, but not creatinine or other meat bases. Saturation with zinc sulphate, plus a little acid, was a not very reliable method for precipitating albumoses and peptones, but sometimes had its uses. Well-made

meat extracts should contain little or no gelatin, except incidentally. The direct addition of this substance was indefensible, except in the tablets or squares. The determination of gelatin by the ice-water method of Stutzer was perhaps the most widely used method, but substances rich in albumose, such as Witte's peptone, yielded appreciable portions of their nitrogen by this method. It was believed that more work needed to be done on the trichloroacetic acid method.

The residual nitrogen or meat bases were estimated from the nitrogen content by the factor of 3.12 (the factor for creatine), but Hefner had suggested 6.25 as better, since it did not deceive with a sense of false accuracy. Creatine and creatinine formed the great bulk of these meat bases. The purine bases were also present in considerable amounts (0.65 to 0.8% of nitrogen in meat extracts and 0.83% in yeast extracts from well known sources).

Tankard centrifuged the bromine precipitate because of the difficulty in filtering. He mixed the residue with bromine water and again centrifuged. He always used zinc sulphate to separate the albumoses and peptones. In most cases it was of little value, but some common preparations were fortified to about 52% of protein with albumoses and peptones. One preparation contained 20% of albumoses, 13% of peptones, and 6% of added meat fibre. He believed that one should distinguish between meat fibre and coagulable proteins. It seemed extraordinary that the brown colour of meat extract solutions should not affect Folin's final red colour in creatinine determinations, but this was a fact. He believed that the factor of 6.25 was a very bad one to use for meat bases and preferred one about 3, say, the factor of 3.12 of Stutzer. For yeast extract this factor was probably too high.

Hinks agreed with Tankard concerning the factor to be used for meat bases.

Barger stated that carnosine had a low factor, whilst some other extractives had a high one. After hydrolysis of proteins the factor was 5.4 and not 6.25. The best value might be between 3 and 5.4, say 4.0.

The Van Slyke method of determining amino acids was thought to be useful.

Walpole stated that the Sørensen formol titration method was a good one for obtaining directly the liberated amino groups.

**Glucose in Meats.**—Beck and Casper (*Z. Unters. Lebensm.*, 1928, 56, 437) have reported on the albuminous compounds from the meat of different animals. Their work has some bearing on meat extracts and on the identification of species (in which connection see also pp. 243 and 450).

Beck and Casper used Striegold's method (*Chem. Ztg.*, 1917, 41, 313) for the separation of glucose, a 2% solution of the sample being coagulated by heating under a reflux condenser for 5 hours and then for a further 30 minutes after the addition of 1% of tartaric acid. The clear solution was neutralised with sodium hydroxide solution, the albumoses precipitated with 10% of a saturated solution of zinc sulphate, and the glucose finally obtained by the addition of a solution of 22.5 grm. of nitrogen-free tannin and 9 c.c. of acetic acid in 100 c.c. of water.

Van Slyke's phosphotungstic acid method was used for the determination of various nitrogen compounds obtained after hydrolysis of the sample of the gluten-tannin precipitate for 5 hours at 135° with six times the amount of 20% hydrochloric acid, the excess of acid being finally removed *in vacuo*, and the solution diluted with water. The distribution of the nitrogen was then calculated from Van Slyke's formulae. Total nitrogen was obtained by the Kjeldahl method.

Beck and Casper point out that there is a close relation between the distribution of nitrogen in the glucose obtained from edible gelatin and from a Liebig's meat extract, and a common origin is suggested. Hydrolysed muscle fibres of the ox, calf, pig, sheep, horse, goose, and cod gave figures which were similar in all cases. Extracts of the meat of these animals were also examined, the total nitrogen, albumose, and glucose being obtained as above, while, in addition, amino acid nitrogen was determined gasometrically by nitrosyl chloride, creatinine by Folin's method, and total phosphorus by titration of a solution of the ash. The results show wide variations. The extract from the cod was high in coagulable nitrogen, albumose, and glucose, but low in creatinin, phosphorus, and amino-acid nitrogen. Horse and pig flesh gave extracts low in glucose.

## POULTRY

The flesh of the common fowl is of two kinds; the light meat is found principally about the breast, and the dark meat chiefly in the

leg and wing muscles. The colour of the dark flesh is, of course, due to the presence of a larger quantity of blood pigment than is present in the lighter flesh, but from an analytical standpoint the chief differences lie in the amounts of fat and cold-water extractives present. The average of a large number of analyses shows that the light meat contains 1.5% fat and the dark meat 6%, and that the light meat contains more extractives than the dark, this being due, in part, to the lower fat content. The tables which follow give more information on this subject.

**Analysis of Poultry.**—The general methods of analysis already given for fresh meats (p. 277 *et seq.*) apply equally well to poultry. In sampling it is advisable to separate the white from the dark meat and to analyse each separately, since otherwise irregular results will be obtained owing to the varying proportion of the two kinds of meat in different individuals and also to the varying percentages of fat in the two kinds of meat. The sampling must be done carefully by hand, the visible fat, skin, and tendons being removed and analysed apart from the muscular tissue if this is desired.

**Composition of Poultry.**—The accompanying table shows the composition of fresh killed poultry, the analyses being conducted according to the methods given on pp. 280 *et seq.* Each analysis was made on a sample derived from three Plymouth Rock chickens, the dressed weight of each being given in the first column. The free fatty acid estimations were made on the abdominal fat, which was taken out, hashed, and rendered at a low temperature and filtered.

Atwater (*U. S. Dept. Agr. Farmer's Bull.* 182, 1903) has reported the composition of various kinds of poultry. Some of the results have been reported in other publications (*U. S. Dept. Agr. Dept. Bull.* 467, 1916; *Off. Expt. Sta. Bull.* 28, *rev.*, 1906). Her results are shown in the accompanying tables. Atwater and Bryant (*U. S. Dept. Agr. Off. Expt. Sta. Bull.* 28, *rev.*, 1906) have reported some additional work. These results are also given in a table following the others (p. 528).

COMPOSITION OF LIGHT AND DARK MEAT OF FRESH-KILLED POULTRY—RICHARDSON

Sample No.	Dressed weight	Kind of meat	Mois- ture %	Ash %	Fat %	Ammoniacal N.			Free acid in fat %	Cold-water extract						
						Total N. %	Method 1. %	Method 2. %		Total solids %	Ash %	Total N. %	Coagu- lable N. %	Albu- minose N. %	Meat base N. %	Acid as lac- tic ac. %
1.	{ 1531 grm. 1224 grm. 1262 grm. }	Light	73.56	1.32	0.72	3.82	0.037	0.013	Avg. { 0.42 }	8.25	1.20	1.155	0.620	0.030	0.506	0.97
		Dark	73.86	1.26	2.08	3.23	0.038	0.010		5.49	1.20	0.661	0.306	0.028	0.344	0.54
2.	{ 1791 grm. 1495 grm. 2081 grm. }	Light	73.69	1.35	1.87	3.85	0.037	0.013	{ 0.27 }	7.59	1.27	1.080	0.538	0.020	0.533	0.95
		Dark	74.35	1.29	4.14	3.38	0.037	0.011		5.14	1.20	0.673	0.293	0.023	0.356	0.55
3.	{ 1825 grm. 2343 grm. 1718 grm. }	Light	72.63	1.32	2.24	3.88	0.038	0.013	{ 0.27 }	8.06	1.21	1.166	0.613	0.026	0.534	0.95
		Dark	72.91	1.24	5.32	3.35	0.037	0.011		5.19	1.16	0.659	0.302	0.027	0.339	0.53
4.	{ 1815 grm. 2047 grm. 2182 grm. }	Light	73.10	1.31	2.51	4.02	0.040	0.012	{ 0.50 }	8.10	1.16	1.200	0.624	0.026	0.548	0.90
		Dark	74.08	1.26	3.87	3.48	0.041	0.010		5.21	1.16	0.683	0.311	0.025	0.350	0.53
Light	{ Max. Min. Av. }	.....	73.69	1.35	2.51	4.02	0.040	0.013	.....	8.25	1.27	1.200	0.624	0.030	0.548	0.97
		.....	72.63	1.31	0.72	3.82	0.037	0.012		7.59	1.16	1.080	0.538	0.020	0.506	0.90
Dark	{ Max. Min. Av. }	.....	73.24	1.32	1.83	3.89	0.038	0.013	.....	8.00	1.21	1.150	0.599	0.025	0.530	0.94
		.....	74.35	1.31	5.32	3.82	0.041	0.011		5.49	1.20	0.683	0.311	0.028	0.356	0.55
Dark	{ Max. Min. Av. }	.....	72.11	1.24	2.08	3.23	0.037	0.010	.....	5.11	1.16	0.659	0.293	0.023	0.339	0.53
		.....	73.86	1.26	3.85	3.36	0.038	0.011		5.25	1.18	0.669	0.303	0.025	0.347	0.54



## MEAT AND MEAT PRODUCTS

## COMPOSITION OF POULTRY

	Water %	Protein %	Fat %	Ash %
Chicken, young				
Meat.....	66.9	22.6	10.1	1.1
Meat, dark.....	70.1	20.8	8.2	1.2
Meat, light.....	70.3	21.9	7.4	1.1
Giblets.....	71.0	19.8	6.4	1.3
Visible fat removed.....	74.5	21.8	2.5	1.1
Liver.....	69.3	22.4	4.2	1.7
Heart.....	72.0	20.7	5.5	1.4
Gizzard.....	72.5	24.7	1.4	1.4
Chicken, broiler				
Meat.....	69.2	21.1	8.8	1.1
Giblets.....	72.8	18.7	6.1	1.3
Capon				
Meat.....	55.8	21.6	22.1	1.2
Giblets.....	63.3	20.5	14.6	1.3
Chicken, other kinds				
Meat.....	63.4	19.4	16.6	1.0
Giblets.....	64.7	18.7	13.7	1.3
Turkey				
Dark meat.....	57.0	21.4	20.6	1.1
Light meat.....	63.9	25.7	9.4	1.3
Giblets.....	56.7	17.7	23.5	1.2
Dark meat cooked.....	53.7	39.2	4.3	2.2
Light meat cooked.....	58.5	34.6	4.9	1.8
Young, edible portion.....	66.1	24.9	8.7	1.3
Young, cooked.....	52.0	27.8	18.4	1.2
Heart.....	68.6	16.8	13.2	1.0
Liver.....	69.6	22.9	5.2	1.7
Gizzard.....	62.7	20.5	14.5	1.1
Duck				
Meat—breast.....	55.5	17.4	26.1	1.0
Breast.....	73.9	22.3	2.3	1.3
Giblets.....	73.2	17.9	5.0	1.8
Duckling				
Meat.....	48.3	13.5	37.9	0.7
Giblets.....	70.2	18.9	8.1	1.6
Green goose				
Meat.....	46.0	15.0	38.3	0.8
Giblets.....	68.7	22.3	7.3	1.4

## COMPOSITION OF POULTRY.—(Continued)

	Water %	Protein %	Fat %	Ash %
Goose				
Meat.....	51.8	16.2	31.5	1.0
Giblets.....	70.0	20.1	8.2	1.7
Gizzard.....	73.8	19.6	5.8	1.0
Liver.....	62.6	16.6	15.9	1.2
Pigeon				
Meat.....	63.2	22.9	12.1	1.4
Giblets.....	68.1	22.2	5.2	2.3
Squab				
Meat.....	56.6	18.5	23.8	1.4
Giblets.....	69.8	19.8	7.2	2.0
Guinea hen				
Meat.....	68.9	23.4	6.5	1.3
Giblets.....	69.9	20.8	7.1	1.3
Pheasant				
Meat.....	70.0	24.7	4.6	1.1
Giblets.....	68.9	20.1	7.2	1.6
Russian pheasant				
Meat.....	70.6	25.7	2.3	1.4
Giblets.....	74.4	21.2	2.2	1.3
Quail				
Meat.....	66.3	25.4	7.0	1.4
Giblets.....	63.0	21.8	6.2	2.3
Preserved poultry meat				
Smoked goose breast, including skin and fat.....	35.7	20.1	38.7	5.5
Smoked goose breast, skin and outer fat removed.....	61.3	26.4	4.4	8.0
Potted turkey.....	56.0	17.2	22.0	3.0
Potted chicken.....	56.1	19.4	20.3	2.5
Canned chicken soup.....	87.1	2.9	3.3	1.6
Canned chicken gumbo soup.....	91.0	2.4	0.2	1.6
Canned boned chicken.....	57.6	27.7	12.8	2.2
Canned sandwich chicken.....	46.9	20.8	30.0	2.6
Canned sandwich turkey.....	47.4	20.7	20.2	2.7
Canned quail.....	66.9	21.8	8.0	1.6
Terrine de foie gras.....	41.3	13.6	38.2	2.6

COMPOSITION OF EDIBLE PORTION OF POULTRY AND GAME—  
ATWATER AND BRYANT

	Number of samples	Water %	Protein %	Fat %	Ash %
Fresh					
Chicken, broilers.....	3	74.8	21.5	2.5	1.1
Fowls.....	26	63.7	19.3	16.3	1.0
Goose, young.....	1	46.7	16.3	36.2	0.8
Turkey.....	3	55.5	21.1	22.9	1.0
Cooked					
Capon.....	1	59.9	27.0	11.5	1.3
Chicken, fricasseed.....	1	67.5	17.6	11.5	1.0

Moulton and Ritchie (*J. Assoc. Off. Agr. Chem.*, 1924, **8**, 158) have reported analyses of the flesh of the squab and pigeon. The flesh of two birds of each age, as well as water extracts, was analysed. The globulin nitrogen is reported in two fractions, A being the globulin soluble in the cold water extract and precipitated by half saturation with zinc sulphate, and B being the globulin soluble in 10% ammonium sulphate solution. These results are shown in the following table.

COMPOSITION OF THE FLESH OF THE SQUAB AND PIGEON—  
MOULTON AND RITCHIE

	Squab %	Pigeon %
Water.....	72.85	72.64
Fat.....	7.52	4.31
Ash.....	1.17	1.29
Phosphorus.....	0.234	0.272
Ether-soluble phosphorus.....	0.020	0.011
Equivalent lecithin.....	0.537	0.314
Total nitrogen.....	2.794	3.466
Water-soluble solids.....	5.10	6.55
Water-soluble ash.....	1.11	1.12
Water-soluble nitrogen.....	0.563	0.795
Globulin A nitrogen.....	0.114	0.214
Albumin nitrogen.....	0.109	0.228
Proteose nitrogen.....	0.031	0.037
Peptone and peptid nitrogen.....	0.111	0.043
Amino acid and extractive nitrogen.....	0.198	0.273
Globulin B nitrogen.....	0.153	0.213

Peterson and Elvehjem (*J. Biol. Chem.*, 1928, **78**, 215), in connection with their work on iron in foods, have reported the following figures for iron in poultry.

## IRON CONTENT OF POULTRY—PETERSON AND ELVEHJEM

	Water %	Iron %
Chicken, dark meat.....	67.5	0.00101
Chicken, light meat.....	76.6	0.00070
Duck.....	43.7	0.00171
Goose.....	57.0	0.00202
Turkey, dark meat.....	72.1	0.00204
Turkey, light meat.....	72.2	0.00103

**Cold Storage of Poultry.**—It is customary in various parts of the world, especially in North America and Russia, to store poultry in freezers at a temperature below  $-9^{\circ}$  from the season of plenty to the season of scarcity. The sanitary and dietetic aspects of this practice have been carefully investigated during the last few years, and the conclusion reached that under proper conditions of storage poultry does not deteriorate when solidly frozen during the usual time of storage. Pennington's experiments lead her to the conclusion that poultry may be kept in good condition for a year (Pennington, *Hearings before U. S. Senate Committee on Manufactures Relative to Foods Held in Cold Storage*, 1911), and Richardson's experiments indicate that the time may be extended to at least three years, and probably longer. More decomposition is developed in a fowl stored at room temperature ( $26^{\circ}$ ) for 24 to 48 hours than when stored below  $-9^{\circ}$  for 5 years. In the latter case decomposition is practically nil. It was formerly considered the best practice to store poultry in the eviscerated condition, but it has been shown that if the viscera are left in place and the fowls chilled and frozen at once, they will reach the consumer in better condition than when eviscerated. (See Pennington, "The Comparative Rate of Decomposition in Drawn and Undrawn Market Poultry," *Circular 70, Bur. Chem. U. S. Dept. Agric.*) The following tables show the composition of poultry stored in frozen condition at  $-9^{\circ}$  for various periods of time. The chickens were Plymouth Rocks of uniform weight killed at the same time and stored in the same place. The figures present some variations, but indicate no progressive change of any kind and, in general, agree closely with the similar figures for fresh-killed chickens.

The abdominal fat of chickens appears to be more susceptible to hydrolysis than fats from some other sources, and it has been thought by some investigators that the percentage of free fatty acids in chicken fat would afford an indication of the length of time

COMPOSITION OF FROZEN POULTRY, AT VARIOUS PERIODS OF STORAGE

Age	Dressed weight	Kind of meat	Moisture %	Ash %	Fat %	Total N. %	Ammoniacal N.		Free acid in fat %	Cold-water extract					Meat base N. %	Acid as lactic ac. %
							Method 1, %	Method 2, %		Total solids %	Ash %	Total N. %	Coagulable N. %	Albuminose N. %		
88 days.	.....	Light	72.93	1.41	0.78	4.13	0.036	0.014	.....	8.69	1.21	1.285	0.671	0.025	0.571	1.00
	.....	Dark	71.95	1.25	5.25	3.93	0.032	0.012	.....	5.58	1.16	0.689	0.310	0.028	0.372	0.07
129 days.	1600 grm.	Light	74.60	1.28	2.05	3.78	0.034	0.011	0.45	7.70	1.13	1.126	0.588	0.028	0.519	0.81
	2040 grm.	Dark	73.21	1.24	5.97	3.34	0.031	0.010	.....	5.27	1.16	0.679	0.379	0.023	0.334	0.50
228 days.	1680 grm.	Light	73.88	1.28	1.20	3.76	0.039	0.012	0.36	7.68	1.13	1.160	0.560	0.030	0.554	0.91
	1445 grm.	Dark	72.34	1.22	5.33	3.45	0.016	0.012	.....	5.61	1.16	0.759	0.380	0.026	0.360	0.52
238 days.	1650 grm.	Light	73.34	1.31	1.08	4.08	0.038	0.012	0.50	7.89	1.15	1.152	0.555	0.022	0.575	0.94
	1705 grm.	Dark	73.81	1.22	4.23	3.45	0.015	0.012	.....	5.81	1.11	0.761	0.369	0.024	0.368	0.66
268 days.	1882 grm.	Light	72.81	1.27	1.45	4.00	0.040	0.013	0.48	8.10	1.16	1.190	0.606	0.031	0.574	0.97
	1655 grm.	Dark	72.61	1.22	4.77	3.48	0.019	0.012	.....	5.49	1.11	0.732	0.358	0.033	0.359	0.54
700 days	2290 grm.	Light	71.97	1.27	2.64	3.90	0.039	0.017	0.62	8.13	1.13	1.158	0.542	0.021	0.589	0.95
	2157 grm.	Dark	69.85	1.18	8.22	3.41	0.036	0.015	.....	5.43	1.06	0.697	0.300	0.031	0.373	0.50
706 days	(2 only)	Light	73.08	1.24	0.72	3.99	0.038	0.016	0.60	8.18	1.12	1.197	0.602	0.028	0.582	0.90
	2245 grm.	Dark	72.90	1.23	4.99	3.43	0.036	0.015	.....	5.52	1.07	0.715	0.332	0.028	0.368	0.56
740 days	1785 grm.	Light	72.00	1.23	1.05	3.94	0.040	0.015	0.55	8.19	1.11	1.185	0.600	0.025	0.564	0.92
	1935 grm.	Dark	72.23	1.23	6.39	3.17	0.036	0.015	.....	5.38	1.04	0.691	0.313	0.032	0.349	0.53
760 days	2230 grm.	Light	71.21	1.19	1.39	3.95	0.041	0.014	0.60	7.91	1.16	1.162	0.577	0.028	0.560	0.90
	1915 grm.	Dark	73.13	1.24	1.39	3.95	0.041	0.014	.....	5.44	1.14	0.710	0.344	0.031	0.361	0.62
Light	1945 grm.	Light	73.14	1.21	4.82	3.45	0.040	0.014	.....	8.69	1.21	1.285	0.671	0.031	0.589	1.00
	Max.	.....	74.60	1.41	2.64	4.13	0.041	0.017	.....	7.68	1.11	1.126	0.542	0.022	0.519	0.81
Dark	Min.	.....	71.97	1.23	0.72	3.76	0.034	0.011	.....	8.05	1.14	1.179	0.589	0.026	0.565	0.92
	Av.	.....	73.11	1.28	1.44	3.95	0.038	0.013	.....	5.81	1.16	0.759	0.380	0.033	0.373	0.67
Average of fresh chickens for comparison.	Max.	.....	73.81	1.25	8.22	3.63	0.040	0.015	.....	5.27	1.04	0.679	0.300	0.033	0.334	0.50
	Min.	.....	69.85	1.18	4.23	3.34	0.032	0.010	.....	5.50	1.10	0.714	0.316	0.028	0.360	0.56
	Av.	.....	70.16	1.21	5.55	3.44	0.035	0.013	.....	8.00	1.21	1.150	0.599	0.025	0.530	0.94
	Light	.....	73.24	1.32	1.83	3.89	0.038	0.013	.....	5.25	1.18	0.669	0.353	0.025	0.347	0.54
	Dark	.....	73.80	1.26	3.85	3.36	0.038	0.011	.....							

## (A) PERCENTAGE COMPOSITION OF FRESH CHICKEN MUSCLE

Sample	Kind of meat	Water %	Fat %	Ash %	Protein (N × 6.25) %	Creatine (N × 3.11) %	Total solids %	Sum of constituents determined %
No. 66. Plymouth Rock broiler	Light	75.50	0.49	1.17	23.46	1.10	24.50	97.92
	Dark	71.75	2.40	1.21	21.40	0.827	28.25	101.33
No. 68. Plymouth Rock young roaster or broiler	Light	75.73	0.17	1.33	21.84	1.01	24.27	100.08
	Dark	75.86	1.38	1.49	21.07	0.64	24.14	100.44
No. 73. Plymouth Rock roaster	Light	73.30	0.51	1.24	22.52	0.920	24.70	100.92
	Dark	74.48	2.88	1.18	20.69	0.743	25.52	99.97
No. 78. Rhode Island Red roaster	Light	73.56	0.98	1.26	23.50	1.01	26.44	100.31
	Dark	73.02	2.99	1.35	23.13	0.64	26.99	101.13
No. 86. Rhode Island Red large broiler	Light	75.01	0.53	1.21	21.95	1.02	24.99	99.13
	Dark	75.94	2.15	1.13	19.77	0.796	24.06	99.78

White, Max.	75.73	0.98	1.33	23.50
Min.	73.30	0.17	1.17	21.84
Dark, Max.	75.94	2.99	1.49	23.13
Min.	71.75	1.38	1.13	19.77

## (B) ANALYSIS OF FAT OF FRESH CHICKEN

Determinations	Plymouth Rock broiler, No. 66	Plymouth Rock broiler, No. 68	Plymouth Rock broiler, No. 73	Rhode Island Red roaster, No. 78	Average	Max.	Min.
Iodine value	62.6	71.2	62.7	61.6	64.4	71	61
Saponification value	188.2	176.7	190.2	176.8	181.4	190	177
Acid value	0.5	1.5	0.8	0.5	0.7	1.5	0.50
Ester value	181.7	175.2	189.4	176.3	180.6	189	175
Free acid, calculated as oleic, %	0.25	0.76	0.40	0.25	0.41	0.76	0.25
Hehner value	86.8	84.25	88.7	85.79	86.36	89	84
Refractive index at 35°				1.4566			

(C) PERCENTAGE OF NITROGEN IN MUSCLE OF FRESH CHICKEN  
Light meat

Determinations	No. 66	No. 68	No. 73	No. 78	No. 86	Average	Max.	Min.
Total nitrogen	4.11	3.82	3.90	4.0	3.84	3.94	4.11	3.82
Total nitrogen in aqueous extract	0.920	0.954	0.995	0.923	0.923	23.91	0.995	0.920
Congulable nitrogen in aqueous extract	22.40 <sup>1</sup>	25.00	25.5	22.65	24.03			
Albumose nitrogen	0.338	0.408	0.534	0.402	0.371	10.42	0.534	0.338
	8.23	10.7	13.7	9.90	9.66			
	0.0260	0.0290	0.0181	0.0188	0.0302	0.675	0.0392	0.018
	0.655	0.775	0.465	0.462	1.02			
Amino acid nitrogen	0.355	0.200	0.206	0.325	0.328	8.27	0.355	0.206
	8.65		7.90	8.00	8.55			
Peptone nitrogen (by difference)	0.200		0.147	0.177	0.185	4.19	0.200	0.147
	4.87		3.76	4.35	4.81			
Nitrogen insoluble in water (by difference)	3.19	2.86	2.90	3.15	2.92			
	77.60	74.8	74.5	77.5	76.00	76.09	3.19	2.86

## (C) PERCENTAGE OF NITROGEN IN MUSCLE OF FRESH CHICKEN

(Continued)

Dark meat

Determination	No. 66	No. 68	No. 73	No. 78	No. 86	Average	Max.	Min.
Total nitrogen.....	3.69	3.58	3.55	3.91	3.42	3.63	3.91	3.42
Total nitrogen in aqueous extract	0.650	0.704	0.639	0.569	0.645	0.637	0.704	0.569
Coagulable nitrogen in aqueous extract	17.85 <sup>1</sup>	19.68	18.00	14.30	18.85	17.73	0.704	0.569
Albumose nitrogen..	0.291	0.320	0.331	0.252	0.262	0.291	0.331	0.252
Amino acid nitrogen	7.89	8.93	9.34	6.45	7.66	8.05	0.331	0.252
Peptone nitrogen (by difference)	0.0235	0.0205	0.0112	0.0103	0.0215	0.0235	0.0235	0.0103
Nitrogen insoluble in water (by difference)	0.637	0.573	0.316	0.263	0.628	0.438	0.637	0.263
	7.21	.....	6.75	5.33	7.48	6.69	0.266	0.208
	0.266	.....	0.239	0.208	0.256	0.266	0.266	0.208
	0.087	.....	0.057	0.090	0.106	0.087	0.106	0.057
	2.36	.....	1.60	2.56	3.1	2.39	0.106	0.057
	3.03	2.88	2.91	3.34	2.77	3.03	3.34	2.77
	82.00	80.5	82.1	85.5	81.3	82.27	85.5	81.3

<sup>1</sup> All figures in bold-faced type are percentages based on total nitrogen.(D) NITROGENOUS CONSTITUENTS OF FRESH CHICKENS  
(CALCULATED ON WATER-ASH-FAT-FREE BASIS)

Light meat

Determinations	No. 66 %	No. 68 %	No. 73 %	No. 78 %	No. 86 %	Average %
Total nitrogen.....	19.74	16.77	16.95	16.80	16.38	17.32
Total nitrogen in aqueous extract.....	4.43	4.19	4.33	3.81	3.94	4.14
Coagulable nitrogen in aqueous extract.....	1.626	1.79	2.32	1.66	1.58	1.77
Albumose nitrogen.....	0.110	0.130	0.078	0.079	0.167	0.112
Amino acid nitrogen.....	1.46	.....	1.29	1.34	1.40	1.37
Peptone nitrogen (by difference).....	0.962	.....	0.639	0.409	0.790	0.70
Nitrogen insoluble in water (by difference).....	15.31	13.97	12.62	12.99	12.42	13.46

Dark meat

Total nitrogen.....	13.90	16.65	16.50	17.25	16.42	16.14
Total nitrogen in aqueous extract.....	2.49	3.27	2.97	2.51	3.10	2.86
Coagulable nitrogen in aqueous extract.....	1.10	1.49	1.62	1.11	1.26	1.31
Albumose nitrogen.....	0.0890	0.0953	0.0472	0.0455	0.101	0.075
Amino acid nitrogen.....	1.008	.....	0.968	0.916	1.23	1.030
Peptone nitrogen (by difference).....	0.328	.....	0.265	0.437	0.509	0.384
Nitrogen insoluble in water (by difference).....	11.42	13.38	13.53	14.74	13.32	13.27

the fowl had been held in cold storage. On the contrary, the percentage of free fatty acids in chicken fat affords a sure indication

of the length of time the bird has been held out of cold storage, either before or after the storage period, since the free acid does not increase appreciably while the bird is solidly frozen during the ordinary period of storage. High fatty acid content indicates storage at the higher temperatures for too long a period, and is, therefore, an excellent indication of improper handling. While no absolute limit can be set, it is safe to say that a free fatty acid content below 1% in the abdominal fat of poultry is indicative of good handling, and one above 3% is evidence of bad handling.

**Analyses of Cold-stored Chickens.**—Pennington (*Bull.* 115, *Bur. Chem., U. S. Dept. Agric.*) reports several analyses of fresh chickens, shown in the above tables, and draws particular attention to the comparatively low acid values shown in Table B. These are as they should be in fresh fatty tissue, the figure 0.76%, calculated as oleic acid from the Plymouth Rock broiler No. 68, being high rather than low. Attention is also directed, in Table C, to the relatively large amount of protein in the light meat as compared with that in the dark, and its greater solubility in water.

Pennington determined moisture by drying 10 grm. at 100° in a tared lead bottle-cap to practically constant weight; fat by extracting the dried residue in Knorr's apparatus for 16 hours with Squibb's ether; ash by charring 2 to 3 grm. in a platinum crucible in a muffle, extracting several times with hot water, filtering, igniting the char and filter, and, after evaporating the filtrate, combining the residues and again igniting in the muffle; total nitrogen by the Kjeldahl-Gunning method; cold-water extract by a complicated modification of the process which seems to have no advantage over that described on p. 292; amino acids by the uncertain tannin and salt method; and creatine by the Folin picric acid method.

## EGGS

According to W. D. Halliburton, the egg of the hen contains, on an average, in 1,000 parts: shell, 106.9; white, 604.2; and yolk, 288.9. (See pp. 542-543.)

Egg shells consist of a keratinoid substance infiltrated with calcium carbonate and traces of calcium phosphate and magnesium carbonate.

The colouring matter is a bile pigment.



### White of Egg

The white of eggs consists of a semi-fluid material of alkaline reaction, contained in a double skin or net-work of firmer fibrous substance, which latter is insoluble in hot or cold water, dilute acetic acid, or solution of common salt. According to Lehmann. the interstitial semi-fluid substance, or white of egg proper, has sp. gr. 1.045 and contains from 85 to 88% of water and an average of 13.3% of solids. Of this, 12.2% consists of proteins, 0.5% of dextrose, 0.66% of ash, with traces of cholesterol, lecithin, fat, alkaline soaps, etc.

Poleck and Weber (*Poggendorff's Annalen*, 79, 155; 81, 91) found the ash of white of egg to contain:  $K_2O$ , 27 to 28%;  $Na_2O$ , 23 to 32;  $CaO$ , 1.7 to 2.9;  $MgO$ , 1.6 to 3.7;  $Fe_2O_3$ , 0.4 to 0.5;  $Cl$ , 25 to 28;  $P_2O_5$ , 3.7 to 4.8;  $SO_3$ , 1.3 to 2.6;  $SiO_2$ , 0.2 to 2.0; and  $CO_2$ , 7 to 9%. Nicklés found a trace of fluorine.

White of egg is a nearly pure solution of proteins, the principal of which is that commonly called egg albumin. The term "albumen" should be limited to its original signification, namely, the white of egg; the word "albumin" being applied to the most characteristic constituent thereof, and extended to other analogous substances contained in blood-serum, etc. The terms "albumen" and "albumin" will then have the same relation to each other as benzol and benzene.<sup>1</sup> A variety of globulin is also present in small quantity and may be separated from the albumin by treating the solution with dilute acetic acid or carbon dioxide, or by saturating it with common salt or magnesium sulphate. The globulins form about 6.7% of the total proteins of egg white. Peptones and albumoses appear to be absent from fresh eggs, but make their appearance in increasing amount as the egg becomes stale. According to E. Salkowski (abst. *J. Chem. Soc.*, 1894, 1, 214), if a solution of hen's egg albumen be carefully neutralised by dilute acetic acid, and the liquid precipitated by boiling, a hitherto unobserved albumose is found in the filtrate, and may be precipitated by concentrating the liquid and adding absolute alcohol.

Ovalbumin is the chief protein constituent of egg-white. It is obtained in the filtrate from the precipitation of the globulins by saturating the solution with ammonium sulphate and allowing the solution to evaporate at a relatively low temperature.

<sup>1</sup> In pronouncing the word albumin, it is correct to accentuate the penultimate, but of late years extensive custom has justified the accentuation of the first syllable.

The percentage composition of ovalbumin is: C, 52.75; H, 7.10; N, 15.51; S, 1.62; P, 0.12; O, 22.90.

When purified, ovalbumin crystallises in well-formed needles that have been obtained to the extent of 50% of the proteins of the egg-white. In a 2.5% aqueous solution coagulation takes place at 64°. With more dilute solutions a higher temperature is required; at 0.5% solution coagulation does not take place at all. With saline solutions the temperatures of coagulation are raised. The specific rotation of ovalbumin is  $[\alpha]_D = -30^\circ$ .

According to Osborne and Campbell (*J. Amer. Chem. Soc.*, 1900, **22**, 422-450) besides ovalbumin and the globulins, other protein constituents of egg-white are *conalbumin* and *ovomucin*.

The non-crystallisable fractions after obtaining ovalbumin have a higher rotation and sulphur content. By heating to 65° conalbumin is obtained. Its specific rotation is  $[\alpha]_D =$  (about)  $-36^\circ$ . Ovomucin is a glycoprotein, which forms about 10% of the protein constituents of the egg-white. It is found in the residue after all other proteins have been removed by heat coagulation.

White of egg, when evaporated to dryness at 60°, yields from 12 to 13% of solid albuminous residue, having a density of about 1.314 and losing 4% of water on further heating to 140°, without thereby becoming insoluble. The residue yields about 7% of ash on ignition, consisting chiefly of sodium chloride and carbonate, with calcium phosphate.

**Commercial Egg Albumin.**—A method of examining this preparation has been published by P. Carles (*J. Pharm. Chem.*, 1897, **6**, 102; abstr. *J. Soc. Chem. Ind.*, 1897, p. 767).

**Egg-albumin.**—When white of egg is beaten up thoroughly with water, the albumin and salts pass into solution, while the insoluble membranous matter may be strained off. The albumin may be partially separated from the soluble salts by dialysis, or by precipitating the liquid with basic lead acetate, decomposing the precipitate by carbonic acid, and removing the last traces of lead by hydrogen sulphide. On very cautiously warming the liquid to 60° incipient coagulation occurs, and the first flakes of albumin carry down with them every trace of lead sulphide, leaving the liquid perfectly colourless. On evaporating the solution at a temperature below 40°, and completing the desiccation in shallow trays, the albumin is obtained in the form of transparent, pale yellow, horny scales, which

may be reduced to a yellowish-white powder. In the solid state it may be kept without change, but the solution readily putrefies.

Albumin so obtained has a sp. gr. of 1.262, and is tasteless, odourless, and neutral in reaction. It dissolves slowly in pure water, but more readily in presence of a little neutral salt or a trace of free alkali. The solution is glairy.

Albumin obtained by dialysing white of egg or blood serum always retains about 1% of mineral matter. An ash-free product has been obtained from white of egg by Hofmeister, but from its method of preparation it can not be regarded as unchanged albumin (see E. Harnack, *Ber.*, 1890, 23, 3745).

The albumin of blood-serum closely resembles egg-albumin, but the two substances are not identical. The differences between them are detailed in Vol. VIII, pp. 625, 643 and 691.

The coagulation of albumin is fully described in Vol. VIII, p. 643.

If very dilute and added in small quantity only, the majority of mineral acids do not precipitate cold albumin solutions; but larger proportions of acid precipitate the albumin completely. Nitric acid acts most strongly, while the precipitate produced by hydrochloric acid is soluble in excess, and on diluting the resulting solution with water a precipitate is produced which, when separated and freed from the mother-liquor, dissolves in water and exhibits the reactions of acid-albumin.

Cold solutions of egg-albumin are not precipitated under ordinary circumstances by carbonic, acetic, tartaric, or orthophosphoric acid, but, in presence of a certain proportion of sodium chloride or other neutral salt, precipitation ensues. Hence, common salt will precipitate a solution of albumin in acetic acid, the precipitate being soluble in pure water if heating has been avoided.

According to F. Blenn (*Z. anal. Chem.*, 1896, 22, 127; abst. *J. Chem. Soc.*, 1896, 1, 658), if white of egg is diluted with water, the precipitated globulins filtered off, and a little formaldehyde added to the filtrate, the albumin will be found to have lost its power of coagulating, and to have undergone conversion into a substance exhibiting reactions distinct from those of any known protein.

**Sugar Content.**—Morner (*Z. physiol. Chem.*, 1912, 80, 430) found that the sugar content, mainly glucose, of egg white varies from 0.3 to 0.5% in the common egg. In eggs of other species the lowest sugar was 0.12, the highest 0.32, and the average of 51 varie-

ties 0.22%. (See also Bierry, Hazard and Ranc, *Compt. rend. Soc. biol.*, 1914, 73, 93.)

### Yolk of Egg

The solids of egg yolk consist chiefly of a mixture of proteins. together with a considerable proportion of fat. Other constituents are small quantities of colouring matter, dextrose, cholesterol, and a very considerable proportion of lecithin (Vol. VII). According to Goble, yolk of egg has the following composition:

	%		%
Vitellin.....	15.8	Cholesterol.....	0.4
Nuclein.....	1.5	Fats.....	20.3
Cerebrin.....	0.3	Colouring matters.....	0.5
Lecithin.....	7.2	Salts.....	1.0
Glycero-phosphoric acid.....	1.2	Water.....	51.8

Paladino and Toso (abst. *Analyst*, 1896, 161) state that egg-fat is used in ointments. They find an iodine value of 81.2 to 81.6, and a saponification value of 18.6. Crystals of cholesterol often separate from the fat.

E. Spaeth (abst. *Analyst*, 1896, 233) gives the following as the analytical characters of the fat of egg yolk:

Sp. gr. at 100° (water at 15° being 1).....	0.881
Iodine value.....	68.48
Reichert-Meissl value.....	0.66
Refractometer reading at 25° (on Zeiss' scale).....	68.5
Melting point of fatty acids.....	36°
Iodine value of fatty acids.....	72.6
Phosphorus content = 0.6% (equivalent to 15.04% lecithin).	

Spaeth proposes to employ these data for ascertaining whether pastry and other flour products have been coloured by yolk of egg, or by saffron, picric acid, etc. It would be a simpler and more certain plan to examine the material directly for such colouring matters.

The ash of the yolk of eggs has the following composition:

	%	%
Na <sub>2</sub> O.....	5.12 to	6.57
K <sub>2</sub> O.....	8.05 to	8.93
CaO.....	12.2 to	13.2
MgO.....	2.07 to	2.11
Fe <sub>2</sub> O <sub>3</sub> .....	1.19 to	1.45
P <sub>2</sub> O <sub>5</sub> .....	63.8 to	66.7
SiO <sub>2</sub> .....	0.55 to	1.40

Lecithin is present, both free and in combination with vitellin, and may be obtained by repeated extraction of the egg yolks with alcohol, and distilling off the alcohol under reduced pressure. Then, after adding ether to the syrupy residue, acetone is used as a precipitant of the lecithin.

Of these constituents, the fats, cholesterol and glycerophosphoric acid are extracted by ether.

F. Jean (*Monit. Scient.*, 1892, 561) gives the following as the average composition of three specimens of egg-yolk:

	%
Water (loss at 110°).....	52.6
Fatty matter (extract by petroleum spirit).....	28.0
Vitellin (including aqueous extract).....	18.0
Ash.....	1.4

The predominating protein of egg yolk is a globulin called vitellin. Albumin is also present in small quantities, together with nuclein, with which last substance the iron of egg yolk is in combination. The proteins found by Valenciennes and Fremy in the yolk of the eggs of fishes, and called by them ichthin, ichthulin, and emydin, probably consisted of mixtures of vitellin with nuclein and lecithin.

**Vitellin** is insoluble in water, and is obtained as a white granular residue on extracting egg-yolk with large quantities of ether. It closely resembles myosin, the globulin of muscle, and may be purified by similar means. It differs, however, from other globulins in being soluble in a saturated solution of common salt. Vitellin may be purified by repeatedly dissolving it in a 10% solution of common salt and precipitating by excess of water. The neutral solution of vitellin in very dilute brine coagulates when heated to 70° to 75°. Vitellin appears to exist in egg yolk in combination or intimate association with lecithin and nuclein.

**Nuclein** closely resembles mucin in its physical characteristics, but contains a notable proportion (1.89 to 2.28%) of phosphorus, and no sulphur. It is probable that numerous varieties of nuclein exist, all being compounds of simple proteins with nucleic acid. (See Vol. VIII, pp. 644, 667, 682 and 686.)

**Plant-vitellin**, extracted by dilute solution of common salt from the seeds of oats, maize, peas, white mustard, etc., agrees in all its characters with egg vitellin.

**Analysis of Commercial Yolk of Egg.**—Salted yolks of eggs, either alone or mixed with borax, are largely employed for dressing hides in the tanning process.

For the analysis of such products, F. Jean (*Monit. Scient.*, 1892, 561; abstr. *J. Soc. Chem. Ind.*, 1892, 11, 941) treats 10 grm. of the sample with a few drops of acetic acid, and evaporates the mixtures slowly, with occasional stirring, at a temperature of 50° to 60°. The drying is completed at 110°, and the residue weighed as *dry extract*, the loss being taken as *water*. The solids are powdered and extracted in a Soxhlet tube with hot petroleum spirit, the solution being evaporated and the residual fatty matter weighed. The residue insoluble in petroleum spirit is freed from the solvent by a current of air and then extracted with boiling distilled water, and the solution thus obtained evaporated. The total ash may be determined in the usual manner, but in presence of borax it is difficult to avoid loss of chlorine. Hence, for the determination of the chlorides Jean recommends that the aqueous extract of the sample should be treated with tannin, and an aliquot part of the filtered liquid concentrated, acidified with nitric acid, and precipitated with silver nitrate. In another portion of the clarified aqueous extract the sulphates, borates, etc., may be determined. Jean gives the following examples of analyses made by the above method:

Analytical data	A %	B %	C %	D %	E %
Water.....	58.54	48.910	52.694	46.60	50.76
Ash.....	18.50	17.468	18.740	16.91	15.13
Oil (petroleum spirit extract).....	14.23	18.840	15.550	18.52	19.78
Vitellin (insoluble).....	14.23	13.840	11.460	13.78	12.87
Aqueous extract.....	10.34	0.942	1.556	1.24	1.46
	100.00	100.000	100.000	100.00	100.00

Saline matters	A %	B %	C %	D %	E %
Normal ash.....	1.10	1.112	1.07	1.112	1.112
Sodium chloride.....	16.71	14.850	17.80	14.420	13.080
Boric acid, etc.....	0.78	1.500	17.80	1.478	0.938

Jean calculates the proportion of *pure yolk* in the samples from the fat, on the assumption that the normal proportion of this is constant at 28%. The *added salts* are deduced from the excess over the normal ash corresponding to the pure yolk present. The *added water* is the excess over the quantity corresponding to the fat (that is, 52.6/28 of the petroleum spirit extract). The "*albumin in excess*" is the difference between the "vitellin" found and that corresponding to the fat present, on the assumption that the ratio between them is constant. In this manner, Jean deduced the following as the composition of the samples of egg yolk in question.

	A %	B %	C %	D %	E %
Pure yolk.....	50.80	67.000	55.00	65.00	70.00
Albumin in excess.....	1.19	0.890	3.01	3.13	1.63
Added salts.....	17.40	14.782	18.74	15.80	14.01
Added water.....	30.52	17.328	23.25	15.98	13.76

In the opinion of Allen, the results of Jean can only be regarded as rough approximations. Phosphorus is a constituent of egg yolk that can be given fairly accurately as a constant; according to Bein (*Ber.*, 23, 423), 1.129 grm. of phosphorus is equivalent to 100 grm. of egg yolk.

**Egg Oil.**—A very complete analysis of the oil of egg yolk has been published by M. Kitt (*Chem. Ztg.*, 1897, 21, 303; abstr. *J. Soc. Chem. Ind.*, 1897, p. 448).

**Colouring Matter of Yolk.**—Barbieri (*Compt. rend.*, 1912, 154, 1726) has shown this to be ovochromin, which decomposes at 270° and is a yellow, hygroscopic powder, soluble in its own weight of water, but insoluble in ordinary organic solvents. It is decolorised by hydrogen peroxide, but alkalies and concentrated acids have no action on it in the cold.

**Composition of Eggs.**—Cook (*Bull.* 115, *Bur. Chem.*, U. S. Dept. Agr., 1908) has determined the weights of eggs, in lots of 1 dozen each, as follows:

Lot No.	Grm.	Lot No.	Grm.
Dozen No. 2.....	669	Dozen No. 7.....	647
Dozen No. 3.....	639	Dozen No. 8.....	647
Dozen No. 4.....	648	Dozen No. 9.....	612
Dozen No. 5.....	670	Dozen No. 10.....	611
Dozen No. 6.....	641	Dozen No. 11.....	623

The average weight per dozen, calculated from the above table, is 640.7 grm. and the average weight per egg 53.4 grm.

Atwater and Bryant (*Bull. 28, Revised, Office Exp. Sta. U. S. Dept. Agric., 1906*) report the following analyses of eggs, uncooked and boiled:

Food material	No. of analyses	Ref-use %	Water %	Protein		Fat %	Total carbohydrates %	Ash %	Fuel value per pound
				N $\times$ 6.25 %	By difference %				
Eggs									
Hens', uncooked: <sup>1</sup>									
Edible portion									
Minimum	60		67.2	11.6	11.4	8.6		0.6	660
Maximum	60		75.8	16.0	17.4	15.1		1.6	910
Average	60		73.7	13.4	14.8	10.5		1.0	720
As purchased		11.2 <sup>2</sup>	65.5	11.9	13.1	9.3		0.9	635
Hens', boiled:									
Edible portion									
Minimum	19		68.6	10.0	10.3	9.1		0.6	575
Maximum	19		79.9	15.6	16.8	14.7		1.1	880
Average	19		73.2	13.2	14.0	12.0		0.8	765
As purchased		11.2 <sup>2</sup>	65.0	11.7	12.4	10.7		0.7	680
Hens', boiled whites:									
Edible portion <sup>3</sup>									
Minimum	11		83.1	11.6	12.3			0.4	235
Maximum	11		87.1	14.8	15.4	0.3		1.0	295
Average	11		86.2	12.3	13.0	0.2		0.6	250
Hens', boiled yolks:									
Edible portion <sup>4</sup>									
Minimum	11		48.4	15.3	15.5	32.2		1.0	1,685
Maximum	11		50.2	16.8	18.0	34.4		1.4	1,745
Average	11		49.5	15.7	16.1	33.3		1.1	1,705

<sup>1</sup> Eggs are difficult to analyse, and the discrepancy between the protein by factor and by difference may be due, in part, to incomplete determination of nitrogen and fat. It is also probable that the factor 6.25 is not correct for eggs. The value of protein by difference is, perhaps, the more nearly correct and has been used in the computation of the fuel value per pound.

<sup>2</sup> Average percentage refuse (shell) in 34 samples.

<sup>3</sup> The ash of the whites of 73 eggs contained 3.3% phosphoric anhydride.

<sup>4</sup> The ash of the yolks of 73 eggs contained 57.2% phosphoric anhydride.

In the uncooked eggs the yolks and whites were mixed and analysed together, whereas in the boiled eggs yolks and whites were analysed separately. Therefore, no direct comparison can be made between the two sets of analyses.

Langworthy ("Eggs and Their Uses as Food." *Farmers' Bulletin 128, U. S. Dept. Agric.*) has compiled the following figures from various sources, showing the composition of eggs of several sorts of domesticated and wild birds, and two varieties of turtles.



## AVERAGE COMPOSITION OF EGGS—LANGWORTHY

	Refuse %	Water %	Pro- tein %	Fat %	Carbo- hydrates %	Ash %	Fuel value per pound calories
<b>Hen:</b>							
Whole egg as purchased...	11.2	65.5	11.9	9.3	.....	0.9	635
Whole egg, edible portion...		73.7	13.4	10.5	.....	1.0	720
White.....		86.2	12.3	0.2	.....	0.6	250
Yolk.....		49.5	15.7	33.3	.....	1.1	1,705
Whole egg boiled, edible portion.....		73.3	13.2	12.0	.....	0.8	765
White-shelled eggs as pur- chased.....	10.7	65.6	11.8	10.8	.....	0.6	675
Brown-shelled eggs as purchased.....	10.9	64.8	11.9	11.2	.....	0.7	695
<b>Duck:</b>							
Whole egg as purchased...	13.7	60.8	12.1	12.5	.....	0.8	750
Whole egg, edible portion...		70.5	13.3	14.5	.....	1.0	860
White.....		87.0	11.1	0.03	.....	0.8	210
Yolk.....		45.8	16.8	36.2	.....	1.2	1,840
<b>Goose:</b>							
Whole egg as purchased...	14.2	59.7	12.9	12.3	.....	0.9	760
Whole egg, edible portion...		60.5	13.8	14.4	.....	1.0	865
White.....		86.3	11.6	0.02	.....	0.8	215
Yolk.....		44.1	17.3	30.2	.....	1.3	1,850
<b>Turkey:</b>							
Whole egg as purchased...	13.8	63.5	12.2	9.7	.....	0.8	635
Whole egg, edible portion...		73.7	13.4	11.2	.....	0.9	720
White.....		86.7	11.5	0.03	.....	0.8	215
Yolk.....		48.3	17.4	32.9	.....	1.2	1,710
<b>Guinea fowl:</b>							
Whole egg as purchased...	16.9	60.5	11.9	9.9	.....	0.8	640
Whole egg, edible portion...		72.8	13.5	12.0	.....	0.9	755
White.....		86.6	11.6	0.03	.....	0.8	215
Yolk.....		49.7	16.7	31.8	.....	1.2	1,655
<b>Plover:</b>							
Whole egg as purchased...	9.6	67.3	9.7	10.6	.....	0.9	625
Whole egg, edible portion...		74.4	10.7	11.7	.....	1.0	695
Fresh-water turtle eggs.....		65.0	18.1	11.1	.....	2.9	778
Sea-turtle eggs.....		76.4	18.8	9.8	.....	0.4	738

Greenlee (*Circular 83, Bur. Chem., U. S. Dept. Agric.*) gives the following figures showing the percentage of shell, yolk, and white in fresh and stale eggs.

The figures for the stale egg plainly show the drying effect which occurs, chiefly in the white, when the egg is kept for some time.

## PERCENTAGE OF SHELL, WHITE AND YOLK IN FRESH AND STALE EGGS—GREENLEE

Experiment number and condition	Weight of eggs gram.	Shell %	Yolk %	White %
Fresh:				
I.....	54.3153	10.13	31.35	58.52
II.....	58.5077	9.57	30.81	59.62
III.....	54.1700	10.83	30.00	59.17
IV.....	61.9017	10.53	29.40	60.07
Average.....	57.2237	10.26	30.39	59.35
Stale:				
I.....	51.3885	11.06	33.12	55.82

Unpublished data of the Missouri Agricultural Experiment Station show that eggs may contain 10.42% of shell and 89.58% of contents. The contents are 60% of white and 40% of yolk. The average composition of 30 samples of eggs is as follows:

	Water %	Protein (nitrogen $\times$ 6.25) %	Fat %	Ash %
White.....	85.42	12.57	.....	.....
Yolk.....	53.92	16.11	27.82	1.56
Composite.....	72.82	13.99	11.13	.....

Fat and ash were not determined in the egg white.

Buckner and Martin and others at the Kentucky Agricultural Experiment Station have reported a series of experiments on the calcium metabolism of the laying hen. Their results show the effect of food upon the weight of the shell and contents and upon the ash. In their first experiment (*J. Biol. Chem.*, 1920, 41, 195) they show that the shell may vary in weight from about 2.8 gm. to 5.9 gm., while the contents may vary from about 35 gm. to 52.4 gm. The ash in the shell varied but moderately, averaging 55.33%, while the proportion of ash in the contents averaged 0.91%.

In another experiment Buckner, Martin, and Peter (*Kentucky Agr. Expt. Sta. Bull.* 250, 1923) reported less variation in the weight of shell and contents. One lot of hens, fed with grit and limestone

in their ration, produced eggs that yielded 5.48 grm. of shell and 44.28 grm. of contents. The contents had 0.077% of lime, 0.023% of magnesia, and 0.49% of phosphorus pentoxide. Later reports show the further effects of ration and season on the eggs (see *Kentucky Agr. Expt. Sta. Bull.* 252, 1924; *Bull.* 291, 1929; *J. Agr. Research* 1928, 36, 263).

Friese (*Z. Nahr. Genussm.*, 1923, 46, 33) has reported the average weight of eggs and percentage distribution of white, yolk, and shell for twelve species of fowl and other birds. In addition to the results shown herewith, he reports the physical composition of eggs of the plover, ousel, sparrow, and canary.

PHYSICAL COMPOSITION OF EGGS—FRIESE

Species	No. of eggs	Weight of egg	% White	% Yolk	% Shell
Hen.....	19	57.12	56.22	31.72	12.06
Goose.....	.....	137.38	53.23	32.72	14.05
Duck.....	8	78.11	50.03	38.02	11.95
Turkey.....	4	92.93	56.72	32.51	10.77
Dove.....	5	21.88	72.35	18.05	9.60
Seagull.....	6	40.40	64.28	26.88	8.84
Guinea fowl.....	2	41.14	44.02	39.38	16.60
Pheasant.....	3	27.03	52.79	37.33	9.88

Hepburn and Katz (*J. Franklin Inst.*, 1927, 203, 835) report the physical and chemical composition of the eggs of the goose and duck, as well as a number of fat constants of the abdominal fat.

Terroine and Belin (*Bull. Soc. Chim. Biol.*, 1927, 9, 1074) report the physical composition of hens' eggs and the chemical composition of the white and yolk. They found that the ration had little or no effect on the composition.

Eakins (*Military Meat and Dairy Hygiene*, Williams and Wilkins, Baltimore, 1924) gives the following figures for the composition of hens' eggs: shell, 10-11%; white, 57%; yolk 31-33%. The contents yielded 64.25-65.5% of water, 10.25-12% of protein, 9.3-10.6% of fat, and 0.9% of ash.

Peterson and Elvehjem (*J. Biol. Chem.*, 1928, 78, 215) give the iron content of eggs as follows:

	Water %	Iron %
Whole egg (edible).....	71.9	0.00252
Egg yolk.....	49.5	0.00760

**Eggs in Commerce.**—Eggs, particularly those of the common fowl, have always been an important article of diet, but recently they have assumed a new importance in commerce, owing to the fact that they are marketed in enormous numbers, and also because they are held in cold storage from the season of plenty to the season of scarcity. In England eggs are received in large numbers from Holland, Russia, New Zealand, Australia, and Canada. They are usually packed in one or more layers in large flat boxes between layers of straw or “excelsior.” In the United States of America it is customary to pack eggs in wooden cases, 30 dozen to the case. Each egg stands on end in a pasteboard cell and is separated from its neighbours by pasteboard partitions. The case is made up of 35 layers or tiers in two divisions of “fillers,” 6 dozen eggs to a layer, or 3 dozen to the filler. Four hundred of these cases, or 120,000 dozen eggs, make one car-load. The eggs are placed in the cold storage rooms in the cases, and the rooms are held at a carefully regulated temperature and relative humidity. The temperature is kept as low as possible without freezing the egg, and the humidity as high as possible without causing a growth of mould on the surface of the egg shells. If the humidity is too low, there will be considerable evaporation of moisture from the interior through the shell, and the egg will lose in weight, and its appearance (when broken) will be altered. On the other hand, if the humidity is too high, a growth of mould will rapidly appear on the shells. It is considered good practice to hold the temperature at about  $1^{\circ}$  to  $-2^{\circ}$  and the humidity at about 80% of saturation at this temperature, as determined by a sensitive sling psychrometer graduated in  $\frac{1}{10}^{\circ}$  F. The humidity maintained differs in different establishments.

The cold storage of eggs in the unfrozen condition is very different from that of meats in the frozen condition. In the former case the growth and activity of bacteria and the activity of enzymes are retarded, but not stopped. When good eggs (April eggs are considered best in North America) are promptly placed in cold storage,

they can be held in excellent condition for about one year. However, cold-storage eggs decompose more rapidly than fresh eggs when they are removed from storage to higher temperatures.

**Changes in Eggs during Cold Storage.**—The most noticeable change in the composition of eggs during storage is a loss of moisture, the principal loss being sustained by the whites. If held too long, the flavour is impaired, although under good conditions this is not noticeable in one year's time by careful comparison with perfectly fresh eggs. Cook (*Bull.* 115, *Bur. Chem., U. S. Dept. Agric.*, 1908) reports the following losses in weight in eggs stored for different periods at 0.5°—a rather high temperature for storage.

LOSS OF WEIGHT PER DOZEN EGGS PLACED IN COLD STORAGE  
MAY 24, 1906, TEMP. 0.5° (33° F.)—COOK

Time of storage	Original weight	Final weight	Loss in weight	
	Grm.	Grm.	Grm.	%
1906				
September 7, 3.5 months.....	623	602	21	3.4
1907				
January 7, 7.5 months.....	611	578	33	5.4
June 17, 12.6 months.....	612	565	47	7.7
October 16, 16.6 months.....	647	582	65	10.0
1908				
January 14, 19.6 months.....	647	582	65	10.0

Under good conditions of storage the loss of weight should not be greater than 5% in twelve months, although in many cases the shrinkage in different warehouses amounts to as much as 10%.

The difference in moisture content between fresh and stored eggs shows itself in another way. When fresh eggs are boiled, a loss in weight occurs, owing probably to the contraction of albumin during coagulation, whereas stored eggs, which have lost weight, gain weight when boiled.

Greenlee (*Circular* 83, *Bur. Chem., U. S. Dept. Agric.*, 1911) has studied the moisture losses of eggs stored at different temperatures. He used white Leghorn eggs obtained from a poultry farm, and held them no longer than 24 hours before commencing the experiments. Those held at 0° (32° F.)—a rather high temperature for storage—

PERCENTAGE CHANGE OF THE MOISTURE CONTENT OF WHITE AND YOLK ON HOLDING AT DIFFERENT TEMPERATURES FOR VARYING PERIODS—GREENLEE

Experiment 196 (0°)			Experiment 197 (0°)			Experiment 356 (0°)			Experiment 357 (0°)		
Age in days	White	Yolk	Age in days	White	Yolk	Age in days	White	Yolk	Age in days	White	Yolk
41	87.42	49.15	43	87.54	48.80	0	88.25	47.15	14	88.75	47.17
76	87.15	49.77	83	86.42	49.25	14	87.59	47.87	21	88.06	47.54
166	86.05	50.25	197	86.30	50.54	35	87.55	43.95	35	87.94	48.58
201	86.19	49.73	208	85.96	50.81	49	87.10	49.35	49	88.16	48.37
266	85.35	50.60	.....	.....	.....	.....	.....	.....	.....	.....	.....

Experiment 363 (11°)			Experiment 355 (18°)			Experiment 334 (21°)			Experiment 336 (26°)		
Age in days	White	Yolk	Age in days	White	Yolk	Age in days	White	Yolk	Age in days	White	Yolk
0	88.24	46.63	0	87.66	47.64	0	87.60	47.17	0	87.75	47.97
14	87.57	47.80	7	86.71	48.96	6	87.09	48.53	7	86.98	49.00
35	87.44	49.39	18	86.11	50.28	13	86.03	49.13	11	86.51	49.72
.....	.....	.....	34	85.99	50.56	19	86.45	49.26	15	86.77	49.78
.....	.....	.....	.....	.....	.....	26	85.16	50.27	19	85.89	50.44
.....	.....	.....	.....	.....	.....	33	85.09	50.19	.....	.....	.....
.....	.....	.....	.....	.....	.....	40	84.74	50.65	.....	.....	.....

were kept in the standard 30-dozen egg crate, while those held at higher temperatures were in pasteboard cartons each holding one dozen. After the fresh eggs had been analysed samples from the lots held at 0° were analysed at intervals of one to three months, and from the lots stored at higher temperatures at intervals of 3 to 10 days. Two dozen eggs were usually taken for a sample.

Greenlee's comments on the figures given in the table are as follows:

"The decrease in moisture in the white appears to be easily accounted for by evaporation to the external atmosphere, but the loss in weight as shown by means of the balance is not sufficient to account for the entire decrease in the percentage of moisture. This fact, together with the increased moisture in the yolk, suggests a transfer of water from white to yolk.

"As far as the results given in the table indicate, it cannot be definitely proved that water passes from the white to the yolk. The same results would be obtained if the white took up solids from the yolk, and the yolk would apparently increase in water, if, during the process of desiccation in the determination of solids in the oven at 100°, some of the solids were volatilised. The two chief constituents in the yolk, aside from water, are fat and protein. A determination of the Reichert-Meissl number of the extracted fat indicates no increase of volatile fatty acids, from which it may be reasonably assumed that there is no volatilisation of fatty substance. Furthermore, an analysis of the dried sample gives the same percentage of nitrogen as the percentage of nitrogen in the fresh sample calculated on the water-free basis, which would show that there is no loss of nitrogenous or protein matter. By a process of mathematical calculation it can easily be shown that solids have not passed from the yolk to the white. Although the yolk has decreased in the percentage of its solids by about the same number of points that the white has increased, nevertheless, since the percentage of solids in the yolk greatly exceeds that in the white, the amount which it would be necessary for the yolk to lose in order to account for the experimental data would be more than sufficient to raise the percentage of solids in the white to the experimental figures, regardless of the fact that there is almost twice as much white as yolk, and even if there were no loss of moisture to the external atmosphere.

"This phenomenon of a transfer of water from the white to the yolk may easily be explained by the simple process of osmosis. The yolk, which contains a very high percentage of solids, is surrounded by a membranous tissue called the vitellin membrane, which in turn is surrounded by the egg white, a liquid much more dilute than the yolk. By osmosis the water passes through the membrane from the more dilute to the more concentrated solution until a constant equilibrium is obtained. In the egg this process continues until the vitellin membrane becomes so weak that it breaks, when the white and yolk begin to lose their identity. This action proceeds with such definiteness that by a process of calculation, knowing the original weight of the egg, the loss in moisture to the external atmosphere can be calculated with surprising closeness to the actual loss as shown by the balance."

The following examples are given:

Example I. No. 334 1-7 (40 days at room temperature).

(Fresh eggs = 59.35% white, 30.39% yolk, 10.26% shell.

561 grm. = original weight of 10 fresh eggs.

$561 \times 59.35\% = 332.95$  grm., original weight of white in 10 fresh eggs.

$561 \times 30.39\% = 170.49$  grm., original weight of yolk in 10 fresh eggs.

$332.95 \times 12.40\% = 41.28$  = solids in white of 10 eggs.

$170.49 \times 52.83\% = 90.07$  = solids in yolks of 10 eggs.

$41.28 \div 15.26\% = 270.52$  = final weight of white.

$90.07 \div 49.40\% = 182.33$  = final weight of yolk.

62.43 grm. = total loss to white (calculated).

11.84 grm. = total gain to yolk ( $182.33 - 170.49$ ).

50.29 grm. = loss to external atmosphere (calculated).

(Experimental loss to 10 eggs as taken from No. 344-7 shows an average of 49.53 grm.)

Example II. No. 336 1-5 (19 days at room temperature).

556.6 grm. = original weight of 10 fresh eggs.

$556.6 \times 59.35\% = 330.34$  grm., original weight of white in 10 eggs.

$556.6 \times 30.39\% = 169.15$  grm., original weight of yolk in 10 eggs.

$330.34 \times 12.25\% = 40.46$  grm., solids in white of 10 eggs.

$169.15 \times 53.02\% = 88.01$  grm., solids in yolk of 10 eggs.



$40.46 \div 14.11\% = 286.8$  grm., final weight of white.

$88.01 \div 49.56\% = 177.6$  grm., final weight of yolk.

43.54 grm. = total loss to white (calculated).

8.40 grm. = total gain to yolk (calculated).

35.14 = loss to external atmosphere (calculated).

35.83 = average loss to 10 eggs as shown by experiment No.

336-5.

Cook (*loc. cit.*) has made numerous analyses of fresh and cold-storage eggs with the results given in the following tables. Estimations were made on whites and yolks, separately, of both boiled and unboiled eggs, but since the separation of whites and yolks of the unboiled eggs was unsatisfactory, the results were calculated to the whole egg. Moisture, ash, ether extract, total sulphur and total phosphorus were determined by the methods of the A. O. A. C. (*Bull. 107, Revised, Bur. Chem., U. S. Dept. Agric.*) Lecithin phosphorus and ether extract were estimated in the same portion of sample. The portion was extracted with absolute alcohol for 8 hours directly into the ether extraction flask and the total phosphorus estimated in the ether-alcohol extract, and calculated as lecithin phosphorus. Total nitrogen was estimated by the A. O. A. C. Kjeldahl-Gunning method and the amino compounds by the tannin and salt method. (*J. Amer. Chem. Soc.*, 1906, **28**, 1485. See also pp. 295 and 297.) Proteose and peptone figures were found by difference.

ANALYSIS OF ENTIRE EGG, UNBOILED, FRESH, AND COLD  
STORAGE<sup>1</sup>—COOK  
Wet basis

Descriptions of sample	Solids %	Ash %	Ether extract %	Total sul- phur %	Total P <sub>2</sub> O <sub>5</sub> %	Total nitro- gen %
Fresh eggs.....	25.28	0.69	.....	.....	.....	1.90
Fresh eggs.....	27.70	0.95	12.89	0.24	0.67	2.01
Storage 3½ mos.....	27.29	0.97	.....	0.16	0.39	1.68
Storage 7½ mos.....	33.56	0.91	.....	0.13	1.00	2.10
Storage 12½ mos.....	24.53	0.91	.....	0.18	0.58	2.09
Storage 16½ mos.....	31.62	1.08	12.69	0.20	0.74	2.23
Storage 19½ mos.....	29.78	1.00	12.15	0.27	0.70	2.26
Maximum.....	33.56	1.08	12.89	0.27	1.00	2.26
Minimum.....	24.53	0.69	12.15	0.13	0.39	1.68

# ANALYSIS OF BOILED YOLKS OF FRESH AND COLD STORAGE EGGS—COOK Wet Basis

Description of sample	Moisture	Ash	Ether extract	Total sulphur	Total P <sub>2</sub> O <sub>5</sub>	Lecithin P <sub>2</sub> O <sub>5</sub>	Nitrogen of egg present as—				
							Total	Coagu- lable	Uncoagu- lable	Proteoses and pep- tones	Amino
	%	%	%	%	%	%	%	%	%	%	
Fresh eggs.....	48.74	1.08	30.41	0.21	1.31	0.90	2.61	2.412	0.108	0.090	0.108
Fresh eggs.....	45.39	1.28	31.80	0.23	1.47	1.025	2.63	2.546	0.084	0.058	0.076
Storage 3 1/2 mos.....	49.02	1.54	31.38	0.20	1.10	0.55	2.50	2.37	0.130	0.064	0.066
Storage 7 1/2 mos.....	50.49	1.34	36.40	0.16	1.90	.....	2.50	2.444	0.136	0.069	0.067
Storage 12 2/3 mos.....	52.09	1.41	29.66	0.173	1.293	0.831	2.39	2.199	0.191	0.112	0.079
Storage 16 2/3 mos.....	64.06	1.92	24.82	0.20	1.35	0.84	2.315	1.117	1.198	1.144	0.054
Storage eggs 19 2/3 mos.....	59.82	1.66	29.19	0.22	1.28	0.83	2.545	0.805	1.686	1.054	0.026

## Calculated to a Water-free Basis

Description of sample	Ash	Ether extract	Total sulphur	Total P <sub>2</sub> O <sub>5</sub>	Lecithin P <sub>2</sub> O <sub>5</sub>	Total P <sub>2</sub> O <sub>5</sub>	Nitrogen of egg present as—				Nitrogen results in per cent. of total nitrogen			
							Total	Coagu- lable	Uncoagu- lable	Prote- oses and pep- tones	Amino	Coagu- lable	Uncoagu- lable	Prote- oses and pep- tones
Fresh.....	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Fresh.....	2.1069	59.3640	0.4097	2.5560	1.7358	68.69	5.9017	4.7054	0.3863	0.1756	0.2107	92.41	7.59	3.45
Storage 3 1/2 mos.....	2.3439	62.0582	0.4312	2.6918	1.8769	69.73	4.8160	4.6021	0.1331	0.0142	0.1389	96.81	3.17	2.88
Storage 7 1/2 mos.....	3.0208	61.5535	0.3923	2.5177	1.0789	50.00	4.9039	4.6489	0.2350	0.1255	0.1295	94.79	5.21	2.57
Storage 12 2/3 mos.....	2.7065	72.5204	0.3232	3.8377	.....	.....	5.1707	4.8900	0.2747	0.1398	0.1353	94.69	5.31	2.70
Storage 16 2/3 mos.....	2.9430	61.0977	0.3611	2.6988	1.7345	64.26	4.9885	4.5899	0.3967	0.2338	0.1649	93.01	7.99	4.68
Storage 19 2/3 mos.....	5.3422	69.0595	0.5565	3.7563	2.3372	62.22	6.4413	3.1080	3.3313	3.1630	0.1593	88.55	51.76	49.42
Storage eggs 19 2/3 mos.....	3.3754	59.3534	0.4473	2.6027	1.6877	64.84	5.1749	1.7588	3.4160	3.3031	0.0559	33.99	66.01	64.99



## CALCULATED TO A WATER-FREE BASIS

Description of sample	Ash %	Ether extract %	Total sulphur %	Total P <sub>2</sub> O <sub>5</sub> %	Total nitrogen %
Fresh eggs.....	2.73				7.51
Fresh eggs.....	3.42	46.54	0.87	2.42	7.26
Storage 3½ mos.....	3.55		0.59	1.43	6.16
Storage 7½ mos.....	2.71		0.39	2.98	6.26
Storage 12½ mos.....	3.71		0.73	2.36	8.52
Storage 16½ mos.....	3.42	40.13	0.63	2.34	7.05
Storage 19½ mos.....	3.36	40.80	0.91	2.35	7.59
Maximum.....	3.55	46.54	0.91	2.98	8.52
Minimum.....	2.71	40.13	0.39	1.43	6.16

<sup>1</sup> Whites and yolks analysed separately and results calculated for total egg.

Cook's figures indicate a decrease in coagulable protein and an increase in the proteoses and peptones in the boiled samples. There are marked irregularities in the figures and confirmation should be had from analyses of uncooked eggs held at  $-1.5^{\circ}$  in comparison with eggs held at room temperature (about  $21^{\circ}$ ).

Pennington and Robertson (*Circular 104, Bur. Chem., U. S. Dept. Agric.*) have studied the enzymes present in hens' eggs, as influenced by storage and report lipase and catalase present. The presence of pepsin, trypsin, reductase, and oxidase, is doubtful.

Dinslage and Windhausen (*Z. Unters. Lebensm.*, 1926, **52**, 288), in discussing the estimation of the freshness of eggs, state that experimental results show that the change in the specific gravity of eggs during storage is determined far less by the duration than by the conditions of storage. An important factor is the moisture content of the surrounding air, the loss in density varying inversely with this magnitude. Without a knowledge of the conditions of storage it is quite impossible to judge even approximately the age of an egg from its specific gravity.

Lythgoe (*Ind. Eng. Chem.*, 1927, **19**, 922) has determined the ammonia content of cold-storage eggs, using the aeration method of Folin (*Z. physiol. Chem.*, 1902, **37**, 161; *J. Biol. Chem.*, 1912, **11**, 532) and employing Nessler's solution as the reagent. He reports the following figures for eggs collected during 5 years.

# AMMONIA CONTENT OF COLD-STORAGE MARKET EGGS— LYTHGOE

Mg. ammonia per 100 grams

Month	Lowest	Lower quartile	Median	Upper quartile	Highest	No. of samples
October.....	1.8	2.46	2.65	2.92	3.8	82
November.....	1.4	2.52	2.86	3.20	4.3	405
December.....	1.9	2.69	2.97	3.24	4.2	374
January.....	2.1	2.85	2.99	3.29	4.3	187
February.....	2.2	2.80	3.28	3.66	4.3	40

Other lots of cold-storage eggs (1,100 samples collected during 5 years) showed extreme variations from 1.4 to 5.5 mg., 95 per cent. of the samples showing between 2.1 and 4.3 mg., and the median and average being practically 3 mg. per 100 grm. of egg.

Jenkins and Pennington (*U. S. Dept. Agr. Bull.* 775, 1919) have studied the commercial preservation of eggs by cold storage. For the months covered by Lythgoe's report they found the following amounts of ammonia: September, 2.50; October, 2.72; November, 2.83; December, 2.86; January, 2.94; February 3.26; and March, 3.35 milligrams per 100 grams of egg. A careful examination of eggs was made to determine (1) the keeping qualities of fresh, heated, sound, dirty, and cracked eggs, (2) the relation of the month of storage to preservation, (3) the efficiency of commercial grading of eggs for cold storage, (4) the analysis of bad eggs developing in commercially packed eggs during storage, (5) the relation of care in initial grading to the development of bad eggs during storage, (6) the rate of evaporation of moisture from eggs, (7) the rate of absorption of moisture by case and fillers, (8) the physical and chemical changes in eggs during storage, and (9) the absorption of foreign flavours during storage.

The losses in weight during storage increased from 0.32-1.00% the first month up to a total of 3.81-5.64% at the end of ten months, depending on the case and the cooler in which they were stored.

They found the ammonia content to be a good index of chemical deterioration. Average results are given above. Initial ammonia percentage content of eggs was as follows:

Firsts	Whole shells	0.0018	0.0019	0.0021	0.0020
	Cracked shells	0.0012	0.0018	0.0014	0.0018
	Whole shells	0.0026	0.0020	0.0020	0.0022
	Cracked shells	0.0010	0.0018	0.0014	0.0021
Seconds and Dirty	Whole shells	0.0011	0.0022	0.0025	
	Cracked shells	0.0016	0.0022	0.0018	

**Effect of Freezing.**—Moran (*Proc. Roy. Soc.*, 1925, 98, B, 436) has studied the effect of low temperature on hens' eggs. He found that the white of an egg, after freezing and thawing, becomes separated into a fluid part and a viscous part. It is the temperature reached, as distinct from the freezing rate, which controls the bulk of the change. Precipitation or coagulation was observed only when the white had been stored in the frozen state for long periods. Thus after 4 months' storage at  $-3^{\circ}$  C. the viscous portion of the white showed indications of coagulation. When egg yolk is frozen within the temperature limits of  $-0.65^{\circ}$  and  $-6^{\circ}$ , normal fluidity is regained on thawing, but if the yolk is kept for some time below  $-6^{\circ}$  it will be found, when thawed, to have changed into a stiff putty-like mass. This occurs even though the yolk is first of all frozen within the labile limits of  $-0.65^{\circ}$  to  $-6^{\circ}$ . The freezing and thawing cycle is also accompanied by permanent changes of volume. Eggs supercooled to  $-11^{\circ}$  do not suffer any visible change, but an irreversible change is indicated by a decrease in volume. The changes of state in frozen yolk appear to be due to the precipitation of lecitho-vitellin, so that resolution does not occur on warming. When cooled below  $0^{\circ}$  eggs quickly lose their fertility, and the experiments cited show that the embryo of the egg dies immediately at about  $-6^{\circ}$  to  $-7^{\circ}$ . The optimum temperature for fertile eggs is about  $8^{\circ}$  to  $10^{\circ}$ . At  $8^{\circ}$  incubations were obtained from eggs up to a limiting storage time of 39 days, whilst at  $16.2^{\circ}$  the maximum time was 29 days.

**Commercial Examination and Classification of Eggs.**—The commercial grading of eggs is based upon a number of points, such as colour, size, form, cleanliness of the shell, and freedom from checks and cracks. The common method of ascertaining the quality of the interior of the egg is known as "candling," from the light originally used for the purpose. According to this method a source of light, such as an incandescent lamp, is placed behind an aperture of slightly less diameter than an egg in a dimly lighted room. The

examiner holds the egg against the aperture, at the same time turning the egg rapidly back and forth so that the light shines through it and, to a certain extent, illuminates the interior of the shell. According to the appearance presented an expert can usually tell at once whether the eggs are of good or poor quality and can separate them into a number of classes which are well known in the egg trade. A few inferior eggs usually escape the candler. The following grades of eggs are generally recognised in the United States:

(1) *Extras* and *first* are fresh-laid, sound eggs with clean shell, of medium or large size. *Extras* are specially selected and graded according to the colour of the shell, some localities preferring a brown and some a white shell.

(2) *Seconds* include all grades not included under (1) and which are not *spots* or *rots*. The following are the principal grades:—

(a) *Undersized* are eggs which would be classed as *firsts*, but for their small size.

(b) *Checks* and *cracks* are eggs whose shells are broken, but whose shell membranes are intact.

(c) *Leakers* are eggs whose shells and shell membranes are both broken.

(d) *Dirties* are eggs whose shells are soiled.

(e) *Weak eggs* have watery whites.

(3) *Spots* are eggs which show a spot in the interior when candled. This may be due to various causes, such as mould, blood ring, developing embryo, etc. These eggs are used for manufacturing purposes, chiefly in the leather industry.

(4) *Rots*.—The name is sufficiently descriptive. They are used for the same purposes as spots.

Other types of eggs, also, are met with by the large handler, such as eggs with brown yolks (these are perfectly sound), “green” eggs, having a greenish white, “musty” eggs, and “sour” eggs. It is the last three kinds which are most apt to escape the candler.

**Frozen Eggs.**—Many eggs arrive at cold-storage warehouses in a cracked, broken, or otherwise damaged condition, and in order to avoid the economic waste which would otherwise ensue, the contents of those that are suitable of these eggs are separated and frozen in tinned containers and thus preserved. The demand for frozen eggs has greatly increased during recent years, so that at the present time many whole eggs are broken and the contents frozen to supply

this trade. There is much to be said in favour of preserving eggs by freezing rather than by storage at temperatures above their freezing points, provided good eggs are used for the purpose.

Pennington (*Circular 98, Bur. Chem., U. S. Dept. Agric.*) has studied the preparation of frozen eggs and devised a working system for handling the manufacture in a sanitary manner. She finds that fresh eggs less than one day old, analysed by the Folin aeration method (p. 327) show 0.0012% N. "White rots" contain 0.0030% N or more; whereas "market seconds" in the producing section in summer time contain on an average from 0.0017 to 0.0022% N. Cracked eggs or "checks," as they are called in the trade, dirty-shell eggs and other seconds are commonly used by egg breakers. No "spot" eggs of any sort are used by reputable breakers. The conclusions are reached that, "properly conducted, the freezing and drying of eggs is an industry which is economically desirable, as long as the centres of egg production and egg consumption are so widely separated, and as long as the poor handling methods bring to the concentrators of the producing sections such enormous numbers of eggs which are wholesome but not available for long hauls."

**Desiccated Eggs.**—There are several preparations of dried eggs on the market. Some of these are made from perfectly fresh sound eggs and are adapted to household use. Others are made from the lower grades of eggs, but still come within the edible class, although of inferior quality.

### Methods of Analysis of Eggs

**General Methods.**—The chemist who is called on to examine eggs or pass judgment on their quality must, of necessity, be an egg expert, since their examination presents rather greater difficulties than that of other food products. He must know the commercial classification and grades and have practice in candling. In many cases the bacteriological examination is a necessity, not so much in the case of shell eggs, as of frozen or dried eggs. The general methods of analysis already given, covering moisture, ash, fat, nitrogen, etc. (pp. 277, 286), are applicable to eggs. The chemical methods of detecting decomposition, such as Eber's sulphide test and estimation of ammoniacal nitrogen are also applicable, but it is generally quicker and equally certain to make use of the sense of smell. *Lecithin* may be estimated by following the ether extraction



by extraction with absolute alcohol and estimating phosphorus in the residue, which is dissolved in a mixture of 6 parts of nitric acid and 1 part of hydrochloric acid for the purpose. The final weighing is made as magnesium pyrophosphate after first precipitating as ammonium phosphomolybdate. *Total phosphorus* may be determined by igniting the sample with a little pure sodium carbonate and sodium nitrate and precipitating by means of ammonium molybdate, after acidifying with nitric acid in the usual way. *Inorganic phosphorus* may be determined as described on p. 304. As eggs deteriorate, as judged by physical means, there is a progressive increase in the ratio of inorganic to total phosphorus.

**Estimation of Lecithin.**—Cohn (*Z. öffent. Chem.*, 1913, 19, 54) suggests the following method:

One to 2 grm. of commercial lecithin preparations, or 5 to 20 grm. of food containing lecithin, are extracted for several hours with two successive portions of 100 c.c. of 96% alcohol, the first extraction at ordinary temperature, the second at the boiling temperature of alcohol, a reflux condenser being used. The residue is ground with sand, extracted once more with alcohol, and then boiled for 2 hours with about 100 c.c. of chloroform. When dealing with fatty substances it is advantageous to extract with chloroform immediately after the cold alcohol extraction. In certain cases the extraction with hot alcohol must be continued for 20 hours in order to extract all the phosphorus compounds. The alcohol and chloroform extract is evaporated, the residue boiled for 2 hours with 100 c.c. of chloroform to separate the lecithin from glyceryl-phosphoric acid and free phosphoric acid, and the solution filtered and evaporated. The amount of phosphorus in the residue is estimated by oxidising with nitric and sulphuric acids, or by igniting it with the addition of magnesium oxide, or a mixture of sodium carbonate and potassium nitrate, precipitating the resulting phosphoric acid with molybdic acid solution and continuing in the usual way.

**Estimation of Salicylic Acid in Preserved Eggs.**—Froidevaux (*J. Pharm. Chim.*, 1915, 10, 18) has pointed out that salicylic acid cannot be determined in preserved eggs by the usual procedure. If a mineral acid is used to liberate salicylic acid, an unfilterable magma results. If the magma be treated with ether to extract the salicylic acid, emulsions form, and fats, lipochromes, lecithin, etc., pass into the solvent. He proceeds as follows:

To 25 grm. of powder or 30 grm. of liquid egg, contained in a 500 c.c. porcelain dish, 250 c.c. of water are added, the mixture stirred, 125 c.c. of 8% sodium hydroxide solution added, and the mixture warmed for 45 minutes on the water-bath. The resulting gelatinous mass is broken up with a glass rod, and the particles washed with water by decantation and on the filter. The filtrate is acidified with hydrochloric acid and 20 c.c. of sodium phosphomolybdate solution added to precipitate the protein. The filtrate from this is extracted with ether in the usual way. The method is sensitive to 0.0023 grm. in 100 grm. of material.

**Distinguishing between the Whites of Hen and Duck Eggs.**—Waterman (*Chem. Weekblad.*, 1913, 11, 120) prepares sera by injecting the white of hen eggs and duck eggs, respectively, into rabbits and drawing off the blood a week after the last injection. These sera are standardised by adding to 0.1 c.c. of the serum 1 c.c. of standard solutions of white of hen and of duck eggs, respectively, ranging in strength from 1/1,000 to 1/80,000. A precipitate is formed at the junction of the liquids. The greatest dilution at which this precipitate is formed is taken as the titre of the serum. The whites of the two kinds of eggs should react only with their respective sera. The sample to be tested is made up to various concentrations and treated as above. When the sample is a mixture, it will react with both sera. One analysis showed 60% of white of duck eggs and 40% of hen egg white.

**Estimation of Albumin.**—Labbé and Maguiso (*Compt. rend.*, 1913, 156, 1415) have proposed a volumetric method in which the egg albumin is precipitated by the citro-picric acid reagent prepared according to Esbach.

**A. O. A. C. Methods.**—Methods for the examination of eggs and egg products have recently been proposed for adoption by the Association of Official Agricultural Chemists (*J. Assoc. Off. Agr. Chem.*, 1926, 9, 56; 1927, 10, 50; 1929, 12, 55). These methods are given below.

**Sampling.**—Where large lots are involved it is better to draw a number of samples for separate analysis. In general, experienced judgment is best.

(a) **Liquid Eggs.**—Mix representative containers of liquid eggs and draw about 300 grm. for analysis. A long-handled dipper or ladle helps in taking the sample. Keep the sample hermetically

sealed in a jar in a cool place. Report the odour and appearance and examine for foreign material.

(b) *Frozen Eggs*.—Take samples by boring with an auger at three widely separated places between the centre and circumference of the can. Note the odour. Collect about 300 grm. of sample. Keep frozen until wanted for the analysis, when the sample should be warmed on a water-bath below 50° C. and mixed.

(c) *Powdered Dried Eggs*.—Secure representative samples or containers. For small packages take entire parcel or parcels. For boxes and barrels remove the top layer for six inches, and then draw small quantities to a total of 300–500 grm. Keep hermetically sealed.

(d) *Flaked and Drum-dried Eggs*.—Secure representative samples or containers as described in (c). Albumin samples are prepared by grinding in a mill to pass a 60-mesh sieve, and whole egg and yolk to pass entirely a 20-mesh sieve, or as fine as is practicable. Keep hermetically sealed.

1. **Total Solids.** *Vacuum Method*.—Drop 2 grm. of dried eggs in a covered metal dish, 55 mm. in diameter and 15 mm. high, having a close-fitting cover, at 98°–100° and weigh them. Dry the sample, with loose cover, to constant weight at 98°–100° C. (approximately 5 hours) in a partial vacuum, 25 mm. or less of mercury. Admit dry air to the oven to obtain atmospheric pressure. Tighten the cover and transfer to a desiccator and weigh when cool. Treat liquid or frozen eggs as above, using 5 grm. of sample. Remove the cover and drive off most of the water on a steam bath. Replace the cover and complete the drying, as above.

*Air-Oven Method*.—An air oven at 112°–117° may be used, drying for about three hours.

2. **Organic and Ammoniacal Nitrogen**.—One grm. of dried egg, or 2–3 grm. of liquid preparation, are placed in a 500 (preferably 800) c.c. Kjeldahl flask, and the nitrogen determined by the Kjeldahl, Gunning, or Kjeldahl-Gunning-Arnold methods.

3. **Fat.** *Acid Hydrolysis Method*.—(a) Liquid eggs. Place 5 grm. of material in a 50 c.c. beaker. Add 10 c.c. of strong hydrochloric acid, mix well, set on a water-bath at 75°–80°, and stir frequently for 15–25 minutes, or until the sample is hydrolysed sufficiently to form a clear solution. Add 10 c.c. of 95 per cent alcohol and cool.

(b) *Dried Eggs*.—Place 2 grm. in a 50 c.c. beaker. Add 2 c.c. of 95% alcohol and stir to moisten all particles, in order to prevent lumping on adding the acid. Add 10 c.c. of hydrochloric acid of sp. gr. 1.125 (or 25 + 13), mix, set on the water-bath, and finish as above.

Transfer the prepared sample to a Röhrig or Mojonier fat extraction apparatus. Rinse the beaker into the extraction tube with 25 c.c. of ethyl ether in 3 portions, and shake the mixture well. Add 25 c.c. of redistilled petroleum spirit (boiling point below 60°) mix, and allow to stand. Draw off the ethereal fat solution through a filter, consisting of a cotton plug in the stem of a funnel, into a weighed 125 c.c. beaker or flask containing some porcelain chips. Re-extract twice with a mixture of 15 c.c. of each solvent. Evaporate the collected extracts on a water-bath and dry in a boiling water-oven to constant weight. This takes approximately 90 minutes. Run a blank on the reagents used.

4. **Lipoids.** (a) *Liquid Eggs*.—Weigh 10 grm. into a 200 c.c. nursing bottle, add 100 c.c. of anhydrous ether, stopper with a softened cork, and shake vigorously. Add five 5 c.c. portions of 95% alcohol, and shake after each addition. (The gradual addition of alcohol with shaking coagulates the proteins in a very fine state.) Centrifugalise and decant the liquid into a 250 c.c. beaker containing some bits of broken porcelain. Wash the neck of the bottle with ether and place the beaker (with the fat solution) on a steam bath. Add 15 c.c. of 95% alcohol to the egg residue in the bottle in such a way as to wash down any particles adhering to the sides and set in a water-bath at 70°–80° for 15 minutes. Shake occasionally with rotation so as to moisten all particles with the alcohol. Cool, add 30 c.c. of ether, stopper, shake 5 minutes, centrifugalise, and decant into the original 250 c.c. beaker. Rinse the bottle neck with ether. Re-extract the residue with two successive portions of 20 c.c. of ether, shake for one minute each time, centrifugalise, and decant into the beaker containing the first extract. Evaporate the combined extracts on the steam bath just to dryness. Place in a boiling-water oven for 5 minutes to drive off the water on the sides of the beaker. Dissolve the dried extract in 15 c.c. of chloroform and filter the solution into a previously dried and weighed flat-bottom platinum dish through a plug of cotton packed in a stem of a funnel. Free any solid extract adhering to the beaker with a glass rod and transfer through the filter into the platinum dish by

means of chloroform from a wash bottle all soluble extract from the beaker bottom and sides. Finally, wash the funnel and stem tip. Evaporate the chloroform on a steam bath (an electric fan hastens the evaporation), and dry the dish and contents in a boiling water oven to constant weight (approximately 90 minutes).

*b. Dried Eggs.*—Put 2 grm. into a funnel having a plug of cotton loosely placed in the stem. Wash with ether 4 or 5 times to extract most of the ether-soluble substances. Collect the washings in a 250 c.c. beaker containing bits of broken porcelain and place on a steam bath. Transfer the residue and cotton to a small glass mortar and allow the ether to evaporate at room temperature. Add 2–3 grm. of precipitated calcium carbonate to the egg residue, grind to a fine powder, and transfer the whole to a 200 c.c. nursing bottle. Wash the mortar, pestle, funnel and funnel-stem tip with ether, and add the washings to the original ether extract. Continue as for liquid eggs.

**5. Lipoid Phosphoric Acid.**—Dissolve the lipoids in 10–15 c.c. of chloroform, add 4% alcoholic potassium hydroxide solution, evaporate to dryness on a steam-bath, and char completely in a furnace at a faint red heat. Cover the dish with a cover-glass, add sufficient dilute nitric acid (1 + 3) to make the solution acid, and filter into a 100 c.c. volumetric flask. Wash the filter and residue carefully, make up the filtrate to 100 c.c., and determine the phosphoric acid by the usual gravimetric or volumetric method. For the volumetric method pipette 20 c.c. of solution into a 250 c.c. beaker, neutralise with dilute ammonia (1 + 3) and then slightly acidify with dilute nitric acid (1 + 3). Set the beaker in a water bath at 45°–50°, and add 15 grm. of ammonium nitrate. When the solution has reached the temperature of the bath, add sufficient ammonium molybdate (previously heated to 45°–50°) to precipitate all phosphate, stir, and heat 30 minutes. Filter off the precipitate on an asbestos mat in a Hirsch funnel and wash with cold water. Transfer the precipitate and filter to a beaker or precipitating vessel, dissolve the precipitate in a small excess of standard alkali, add a few drops of phenolphthalein indicator, and titrate with standard acid. The alkali may be sodium or potassium hydroxide of such a strength (323.81 c.c. of 1 N per litre) that 1 c.c. is equivalent to 1 mg. of phosphorus pentoxide. The acid may be hydrochloric or nitric acid and may be as strong, or half as strong, as the alkali.

**6. Ash.**—Two–4 gram. of liquid or 1 gram. of powdered substance are weighed into an ashing dish, preferably a flat-bottomed platinum dish 5 cm. in diameter. A porcelain dish or porcelain crucible of 20–25 c.c. capacity may be used, but not a silica dish. Add 5 gram., accurately weighed, of previously ignited 60-mesh alundum (crystallised alumina), mix, add from a pipette 5 c.c. of a water solution containing 1.5 gram. of magnesium acetate per 100 c.c. Run a blank determination on the magnesium acetate solution. Place the dish on an asbestos-centred gauze and evaporate carefully over a small Bunsen flame. Ignite carefully without removing the gauze, and then heat the dish in a muffle at a low red heat for 2 hours. Cool, weigh, break up the ash with a stirring rod, moisten with 5 c.c. of water, evaporate to dryness on a steam bath, and heat in a muffle for 1 hour. Repeat until no loss of weight results. Subtract the weight of the alundum added and the blank on 5 c.c. of the magnesium acetate solution.

**7. Unsaponifiable Matter.**—Determine the unsaponifiable matter in the extracted lipoids as follows (*J. Assoc. Off. Agr. Chem.*, 1926, 9, 45):

*Reagent.*—Petroleum spirit redistilled below 75°. Run a blank on 350 c.c. of the reagent with about 0.25 gram. of stearine or other hard fat (previously brought to constant weight by heating) and drying, as in the actual determination. The blank must not exceed a few mg.

*Apparatus.*—Extraction cylinder, a glass stoppered cylinder graduated at 40 c.c., 80 c.c., and 130 c.c. and with a diameter of 1 $\frac{3}{8}$  inches and height of 12 inches.

*Procedure.*—Weigh 5 gram. of prepared sample into a 200 c.c. Erlenmeyer flask, add 30 c.c. of redistilled 95% (approximately) by volume ethyl alcohol and 5 c.c. of a 50% water solution of potassium hydroxide, and boil the mixture for one hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 c.c. mark with redistilled 95% alcohol. Complete the transfer, first with warm and then with cold water, until the volume is 80 c.c. Cool the cylinder and contents to room temperature and add 50 c.c. of petroleum spirit. Shake as vigorously as possible for one minute and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 c.c. Draw off the petroleum spirit by means of a slender syphon into a separatory

funnel of 500 c.c. capacity. Repeat the extraction six more times, using 50 c.c. of petroleum spirit each time. Wash the combined extract into the separatory funnel three times with 25 c.c. portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum spirit extract to a weighed Erlenmeyer flask, distil, or, if desired, evaporate, the petroleum spirit on a steam-bath in a current of air. Heat the flask with the residue until a constant weight is obtained in an oven at a uniform temperature of no less than 100°, and no more than 110°. A vacuum oven may be used at corresponding temperatures which depend on the pressure. Displace any residual solvent with air. Deduct any blank obtained from the weight found. Test the residue for solubility in 50 c.c. of petroleum spirit, filter and wash, if there is a residue. Then evaporate and dry as before.

**8. Water-soluble Protein Nitrogen Precipitated by 40 Per Cent Alcohol.** (a) *Powdered Material.*—Two grm. of powdered material is placed in an eight-ounce nursing bottle, 25 c.c. of ethyl ether are added, stopper with a water-soaked cork, shake for several minutes, centrifugalise until the supernatant liquid is clear, and decant, allowing none of the egg to be carried over. Repeat three times more with 20 c.c. portions of ether. Wash off the neck of the bottle with ether after each decantation to remove adhering fat. Dry the fat-free residue by aid of suction and reduce to a fine powder by working with a glass rod. Add slowly 100 c.c. of water from a pipette, shake vigorously to avoid lumping, and add exactly 100 c.c. more of water. Shake the stoppered bottle mechanically or by hand for one hour. The temperature of the water must not exceed 30°.

(b) *Liquid Material.*—Ten grm. of liquid egg is placed in an eight-ounce nursing bottle. Add 100 c.c. of dry ether, stopper with a water-soaked cork, and shake well. Centrifugalise lightly and carefully; decant the ethereal solution, allowing no solids to be carried over. Add 50 c.c. of ether twice more and shake; centrifugalise and filter each time. Dry the fat-free residue with the aid of suction. Add slowly 100 c.c. of water from a pipette and finish as above.

*Determination.*—Centrifugalise the watery solution to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. The filtrate should be practically clear. Pipette off 50 c.c. of filtrate into a

200 c.c. nursing bottle. Add 0.6 gm. of sodium chloride and dissolve. Add 0.2 gm. of ignited asbestos, shake, and, with constant agitation, add 35 c.c. of 95% alcohol. Leave overnight and then centrifugalise to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off and wash with two 20 c.c. portions of 40% alcohol, in each case shaking, centrifugalising, and decanting. If the liquid is not free from suspended matter, filter through a thin asbestos pad (0.1–0.15 gm.) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos in the nursing bottle to a Kjeldahl flask with a stream of water. Add the mat in the Gooch crucible and determine the nitrogen in the material in the Kjeldahl flask.

**9. Acidity of Fat.**—(*Not for egg white.*)

**Reagents.**—Anhydrous ether; benzene, the best and neutral; 0.05 *N* sodium ethylate—dissolve a piece of metallic sodium, approximately 1 c.c. in volume, in 800 c.c. of ethyl alcohol. Titrate 10 c.c. of 0.1 *N* hydrochloric acid with this solution, and add the calculated amount (volume) of absolute alcohol to make the solution 0.05 *N*. Standardise against 0.1 *N* hydrochloric acid on the day the solution is to be used.

**Dried Eggs.**—Into an aluminium dish, 63 mm. in diameter ( $2\frac{1}{2}$  inches), weigh out 2 gm. of material and dry at 55° and under reduced pressure (125 mm. of mercury). Weigh to the third decimal at the end of 2 hours and repeat at  $\frac{1}{2}$  hour intervals until no further loss in weight is shown. Extract the dried residue with anhydrous ether, preferably in a Knorr apparatus. Carefully transfer the egg powder to a 12.5 cm. hardened filter paper, fold the paper once, place it on a 15 cm. qualitative filter paper, and roll the papers and contents into a cylinder that will fit snugly into the extraction tube, folding in one end of the cylinder to prevent loss of material. (An asbestos plug is not needed in the extraction tube and, if the extractor is working rapidly, 3 hours is sufficient to insure proper extraction.) Evaporate the ether from the extraction flask, dry the extract for 1 hour at 55° and under reduced pressure (125 mm. of mercury), and weigh to the third decimal place. Dissolve the extract in 50 c.c. of benzene, add 3–4 drops of phenolphthalein indicator, and titrate with standard sodium ethylate. The end-point is reached when the yellow colour changes to orange. Express the results as c.c. of 0.05 *N* sodium ethylate per gm. of ether extract.



**Liquid Eggs.**—Weigh to the third decimal place in a weighed lead dish 5 grm. of the sample and dry as above. Weigh after drying for about 5 hours, and thereafter at 1 hour intervals until there is no further loss. To prepare the dried residue for extraction with ether, place the lead dish upon a 12.5 cm. hardened filter paper, cut the sides of the dish through four equidistant points, and flatten down. Place another similar filter paper on top of the lead dish and its contents and roll the papers and dish into a cylinder that will fit snugly into the extractor, folding in one end of the cylinder to prevent any egg residue from dropping into the extraction flask. Then proceed as above.

**Preserved Eggs:**—For chemical methods of detecting eggs preserved by means of water-glass or lime, see J. R. Nicholls, *Analyst*, 1931, 56, 383.

### Bacteriological Examination

**Bacteriological Examination of Eggs.**—G. W. Stiles, Jr., has made a bacteriological examination of 10 fresh and 8 cold-storage eggs in connection with the work of Cook (*loc. cit.*), using the following method. After washing the eggs for a few minutes in a 1 to 1,000 mercuric chloride solution or 5% carbolic acid, they were dried with sterile absorbent cotton and placed, large end uppermost, in a small beaker. The air space was then scorched for a few seconds with a gas flame, and an opening immediately made into the cavity with sterile forceps, after removing a sufficient amount of shell without rupturing the membrane below. The membrane was then broken with a hot platinum spatula and 0.5 c.c. of the white quickly removed with a sterile pipette and placed in the usual Petri dishes. The remaining white was decanted leaving in the shell the unbroken yolk. A small portion of the yolk was then removed for examination with another sterile pipette. Difficulty was experienced in breaking up the respective layers of the egg after the samples were taken. The eggs were held in 0.5° (33° F.) storage, and naturally all the changes including increase in bacteria occurred more rapidly than would be the case at a lower temperature. See tables on p. 567.

Stiles, in collaboration with Bates, has also made other and more extensive bacteriological studies of eggs ("A Bacteriological Study of Shell, Frozen, and Desiccated Eggs; Made under Laboratory Conditions at Washington, D. C.," *Bull. 158 Bur. Chem. U. S. Dept.*

## BACTERIOLOGICAL EXAMINATION OF FRESH EGGS—STILES

No. of sample	Received for examination	Portion of egg	Number of colonies per c.c. developing on lactose agar at 35° to 37°	Number of colonies per c.c. developing on lactose gelatin at 12° to 15°	Species of bacteria isolated
4067.....	5/24/1906	White	1	0	<i>Mic. cinnabareus</i> , Flügge
		Yolk	190	3	<i>Myobacterium avium</i> , <i>Streptothrix farcinica</i> , <i>Sarcina lutea</i>
4068.....	5/24/1906	White	0	0	
		Yolk	0	0	
4069.....	5/24/1906	White	1	1	<i>Mic. cumulatus</i> ?
		Yolk	0	0	
4070.....	5/24/1906	White	1	1	<i>Bact. haematoides</i>
		Yolk	240	3	<i>Micrococcus</i> , not classified
4071.....	5/24/1906	White	0	0	
		Yolk	5	0	<i>B. mesentericus</i>
4072.....	5/24/1906	White	0	0	
		Yolk	6	0	<i>Saccharomyces</i> , <i>B. Pammeli</i>
B. C. 551.....	10/17/1907	White	1	0	1 fungus
		Yolk	2	1	1 fungoid colony
B. C. 552.....	10/17/1907	White	0	0	
		Yolk	1	0	1 gray fungus
B. C. 669 <sup>1</sup> .....	1/16/1908	White	0	0	
		Yolk	1	0	<i>Actinomyces</i>
B. C. 670.....	1/16/1908	White	0	0	
		Yolk	0	0	

<sup>1</sup> Gelatin plates incubated at 15° to 20°.

## BACTERIOLOGICAL EXAMINATION OF COLD-STORAGE EGGS

No. of sample	Duration of cold storage	Portion of egg	Number of colonies per c.c. developing at room temperatures
4193.....	3 months	White	6,250
		Yolk	3,370
4195.....	3 months	White	1,280
		Yolk	3,260
4282.....	7.5 months	Total egg	0
4283.....	7.5 months	Total egg	1
B. C. 55.....	11.8 months	Total egg	1 fungus
550.....	11.8 months	Total egg	White fungus at ice-box temperature
B. C. 667.....	1 yr. 7 months 22 days	White	1 colony
		Yolk	1 colony
B. C. 668.....	1 yr. 7 months 22 days	White	0
		Yolk	0

*Agric.*, 1912). The following tables give the results of these studies:

STRICTLY FRESH SHELL EGGS APRIL TO OCTOBER, 1911—  
STILES AND BATES

Number of organisms per c.c., and month	Number of samples of—		Percentage of samples of—	
	Yolk	Albumin	Yolk	Albumin
<b>April, 1911:</b>				
0.....	37	58	39.78	62.36
1-10.....	44	27	47.31	29.03
10-25.....	6	4	0.45	4.30
25-50.....	2	3	2.15	3.22
50-100.....	2	0	2.15	0.00
100-250.....	2	1	2.15	1.07
250-500.....	0	0	0.00	0.00
500 and higher.....	0	0	0.00	0.00
<b>May, 1911:</b>				
0.....	5	16	14.28	45.71
1-10.....	9	13	25.71	37.14
10-25.....	5	3	14.28	8.57
25-50.....	5	1	14.28	2.85
50-100.....	2	1	5.71	2.85
100-250.....	3	1	8.57	2.85
250-500.....	0	0	0.00	0.00
500 and higher.....	6	0	17.14	0.00
<b>June, 1911:</b>				
0.....	5	25	6.25	30.12
1-10.....	16	45	20.00	54.21
10-25.....	23	8	28.75	9.63
25-50.....	14	4	17.50	4.82
50-100.....	14	0	17.50	0.00
100-250.....	2	1	2.50	1.20
250-500.....	2	0	2.50	0.00
500 and higher.....	4	0	5.00	0.00
<b>July, 1911:</b>				
0.....	9	38	6.52	27.53
1-10.....	24	60	17.39	43.47
10-25.....	24	20	17.39	14.49
25-50.....	17	11	12.32	7.97
50-100.....	26	3	18.84	2.17
100-250.....	15	1	10.87	0.72
250-500.....	8	2	5.79	1.44
500 and higher.....	15	3	10.87	2.17
<b>August, 1911:</b>				
0.....	7	20	5.65	16.12
1-10.....	22	67	17.74	54.04
10-25.....	3	10	2.42	8.07
25-50.....	10	12	8.07	9.67
50-100.....	16	10	12.90	8.07
100-250.....	31	5	25.00	4.03
250-500.....	17	0	13.71	0.00
500 and higher.....	18	0	14.51	0.00
<b>September, 1911:</b>				
0.....	5	8	16.66	26.66
1-10.....	3	18	10.00	60.00
10-25.....	5	4	16.66	13.33
25-50.....	8	0	26.66	0.00
50-100.....	3	0	10.00	0.00
100-250.....	3	0	10.00	0.00
250-500.....	2	0	6.66	0.00
500 and higher.....	1	0	3.33	0.00
<b>October, 1911:</b>				
0.....	10	19	8.85	16.81
1-10.....	15	75	13.28	66.39
10-25.....	19	10	10.81	8.85
25-50.....	16	4	14.16	3.53
50-100.....	19	4	16.81	3.53
100-250.....	18	1	15.95	0.88
250-500.....	6	0	5.31	0.00
500 and higher.....	10	0	8.85	0.00

## SUMMARY OF RESULTS—STILES AND BATES

Month	Number of samples	Minimum count		Maximum count		Average count		Percentage of sterile samples	
		Yolk	Albumin	Yolk	Albumin	Yolk	Albumin	Yolk	Albumin
April.....	93	0	0	180	140	6.2	3.6	39.78	62.36
May.....	35	0	0	2,750	120	318.6	10.1	14.28	45.71
June.....	83	0	0	17,500	220	335.1	6.74	6.25	30.12
July.....	83 <sup>1</sup>	0	0	18,000 <sup>2</sup>	900	473.2	36.5	6.52	27.53
August.....	124	0	0	9,300	200	344.2	18.9	5.65	16.12
September.....	30	0	0	1,400	20	99.66	3.6	16.66	26.66
October.....	113	0	0	6,000	328	240.2	9.3	8.85	16.81
Total and average.	616					271.7	15.9		

<sup>1</sup> Three samples of yolk were broken.<sup>2</sup> This is the individual yolk containing *B. coli*.

## STRICTLY FRESH WHOLE EGGS, FROZEN AND EXAMINED AT INTERVALS FROM FEB. 14, 1910, TO MAR. 29, 1911

(Six dozen eggs used, none over 4 days old. *B. coli* not present in 1 c.c. quantities)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2167.....	300	300	0	0
2176.....	400	400	0	1
2213.....	1,200	1,400	0	5
2234.....	1,000	1,000	0	11
2250.....	900	700	0	19
2299.....	1,000	800	0	30
2308.....	500	400	0	37
2376.....	800	800	0	45
2396.....	600	600	0	50
2429.....	1,400	1,300	0	56
2435.....	1,600	1,200	0	63
2455.....	1,000	900	0	71
2556.....	900	900	0	80
2583.....	1,400	700	0	87
2592.....	1,200	1,200	0	92
2621.....	1,700	2,000	0	100
2643.....	300	100	0	109
2659.....	300	300	0	115
2769.....	600	400	0	185
2826.....	100	100	0	197
2906.....	600	200	0	210
2931.....	80	50	0	219
2962.....	700	700	0	253
3038.....	300	400	0	276
3112.....	400	200	0	286
3215.....	100	100	0	323
3366.....	200	200	0	407
Maximum average count.....				1,850
Minimum average count.....				56

**FROZEN ALBUMEN FROM STRICTLY FRESH EGGS, EXAMINED AT  
INTERVALS FROM FEB. 15, 1910, TO MAR. 29, 1911**  
(Four dozen eggs, none over 4 days old)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2168 .....	0	0	0	0
2177 .....	20	10	.....	1
2214 .....	100	100	.....	4
2251 .....	200	100	.....	14
2300 .....	300	200	0	29
2308 .....	400	400	.....	36
2377 .....	160	150	.....	44
2397 .....	200	100	.....	49
2557 .....	600	600	.....	79
2622 .....	500	500	.....	99
2644 .....	100	100	.....	106
2770 .....	200	100	0	182
2826 .....	100	100	.....	194
2907 .....	100	100	.....	206
2932 .....	70	90	.....	216
2963 .....	180	100	.....	240
3040 .....	200	100	.....	262
3113 .....	60	60	0	272
3224 .....	100	100	.....	323
3373 .....	8	7	.....	410
Maximum average count.....	.....	.....	.....	600
Minimum average count.....	.....	.....	.....	0
<i>B. coli</i> not present in 1 c.c. quantities.				

**FROZEN ALBUMEN FROM COMMERCIAL EGGS, EXAMINED AT  
INTERVALS FROM FEB. 16, 1910, TO MAR. 29, 1911**  
(Four dozen eggs bought on the market as fresh)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2189 .....	60	40	0	0
2253 .....	400	300	.....	14
2302 .....	700	500	0	29
2311 .....	600	500	.....	36
2379 .....	700	500	.....	44
2772 .....	400	200	0	182
2827 .....	100	100	.....	194
2934 .....	300	300	0	216
2965 .....	100	100	.....	240
3037 .....	100	100	.....	262
3115 .....	180	100	0	272
3225 .....	100	100	.....	323
3374 .....	20	10	.....	410
Maximum average count.....	.....	.....	.....	600
Minimum average count.....	.....	.....	.....	15
<i>B. coli</i> not present in 1 c.c. quantities.				

COMMERCIAL WHOLE EGGS, FROZEN AND EXAMINED AT INTERVALS FROM FEB. 16, 1910, TO MAR. 29, 1911  
(Eight dozen eggs said to have been in cold storage for 8 months)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2188	800	700	(1)	0
2215	900	900		3
2252	1,400	1,000		14
2301	3,000	2,700	0	29
2311	600	500		36
2378	1,200	1,000		44
2398	2,500	1,300		49
2431	4,400	3,000		55
2436	2,700	2,400		62
2457	2,800	1,000	(1)	70
2558	12,000	7,000		79
2585	1,500	1,400		86
2593	2,200	2,000		91
2623	3,000	1,000		99
2645	7,000	7,000		106
2661	2,000	2,000		112
2771	900	800	(1)	182
2831	1,400	400		194
2908	500	300		206
2933	200	100		216
2964	800	800		240
3030	100		(1)	262
3114	800	400	(1)	272
3223	900	1,100		321
3367	900	800		406

(1) *B. coli* absent in 0.1 c.c.

Maximum average count..... 9,500  
Minimum average count..... 100

EGGS CALLED CHECKS AND CRACKS FROZEN AND EXAMINED AT INTERVALS FROM MAY 12, 1910, TO MAR. 29, 1911  
(Six dozen eggs as received by a commercial egg house among current receipts)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2578	17,000	13,000	(1)	0
2626	200,000	20,000		13
2663	140,000	120,000		27
2777	7,000	4,000	(1)	96
2833	30,000	40,000		108
2915	90,000	60,000		120
2966	34,000	31,000		164
3043	70,000	27,000		186
3116	30,000	21,000	(1)	196
3220	10,000	4,000		262
3369	16,000	15,000		317

(1) *B. coli* not present in 0.1 c.c.

Maximum average count..... 130,000  
Minimum average count..... 5,500

## EGGS CALLED "DIRTIES" FROZEN AND EXAMINED AT INTERVALS

FROM MAY 12, 1910, TO MAR. 29, 1911

(Six dozen eggs as received in current receipts by commercial trade)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2579.....	200,000	140,000	(1)	0
2627.....	170,000	100,000	.....	13
2664.....	320,000	.....	.....	27
2778.....	80,000	20,000	(2)	96
2834.....	100,000	3,200	.....	108
2912.....	160,000	80,000	.....	120
2936.....	90,000	30,000	.....	130
2967.....	100,000	40,000	.....	164
3042.....	18,000	.....	.....	186
3221.....	57,000	14,000	.....	261
3370.....	50,000	40,000	.....	317

(1) *B. coli* not present in 0.1 c.c.(2) *B. coli* not present in 0.01 c.c.

Maximum average count..... 320,000

Minimum average count..... 18,000

"HOT WEATHER" EGGS<sup>1</sup> FROZEN AND EXAMINED AT INTERVALS

FROM AUG. 18, 1910, TO MAR. 29, 1911

(Four dozen eggs received by a commercial house from current receipts)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2770.....	700	200	(2)	0
2913.....	1,000	1,200	.....	25
2937.....	1,400	1,000	.....	36
2968.....	5,000	5,000	.....	68
3039.....	600	900	.....	90
3222.....	700	1,000	.....	137
3368.....	400	300	.....	221

<sup>1</sup> By this commercial term was meant rollers or floaters and eggs with watery albumen.(2) *B. coli* not present in 0.01 c.c.

Maximum average count..... 5,000

Minimum average count..... 350

"LIGHT SPOT" EGGS<sup>1</sup> FROZEN AND EXAMINED AT INTERVALS

FROM MAY 12, 1910, TO JAN. 4, 1911

(Four dozen eggs received by a commercial firm)

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2580.....	6,700,000	2,000,000	100,000	0
2647.....	28,000,000	20,000,000	.....	23
2774.....	2,500,000	3,000,000	100,000	98
2835.....	10,000,000	4,400,000	.....	110
2969.....	8,000,000	6,000,000	.....	166
3041.....	4,300,000	2,900,000	.....	189
3216.....	5,000,000	4,000,000	.....	236

<sup>1</sup> In this grade were included all of the eggs that before the candle showed the beginning of spot development which may have been a stuck spot, an embryo spot, blood ring, or a mould spot.

Maximum average count..... 24,000,000

Minimum average count..... 2,750,000

**"HEAVY SPOT" EGGS<sup>1</sup> FROZEN AND EXAMINED AT INTERVALS  
FROM MAY 12, 1910, TO MAR. 29, 1911.  
(Seven dozen eggs received from the trade as discards)**

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2581.....	22,000,000	6,000,000	1,000,000	0
2595.....	90,000,000	40,000,000	.....	7
2628.....	150,000,000	150,000,000	.....	13
2648.....	150,000,000	110,000,000	.....	21
2775.....	80,000,000	50,000,000	1,000,000	96
2832.....	78,000,000	70,000,000	.....	108
2914.....	90,000,000	70,000,000	.....	120
2938.....	90,000,000	67,000,000	.....	130
2970.....	120,000,000	34,000,000	.....	164
3177.....	180,000,000	100,000,000	.....	262
3371.....	120,000,000	120,000,000	.....	317

<sup>1</sup> In this grade is included all eggs showing a marked development of stuck, mould, embryo spots or blood rings.

Maximum average count..... 150,000,000

Minimum average count..... 14,000,000

**EGGS CALLED BLOOD "RINGS"<sup>1</sup> FROZEN AND EXAMINED AT  
INTERVALS FROM MAY 12, 1910, TO JAN. 4, 1911  
(Four dozen eggs received from the trade as discards from candling)**

Sample No.	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2582.....	500,000,000	300,000,000	.....	0
2649.....	2,160,000,000	2,000,000,000	.....	22
2776.....	800,000,000	500,000,000	.....	96
2829.....	310,000,000	380,000,000	.....	108
2971.....	800,000,000	800,000,000	.....	164
3044.....	675,000,000	600,000,000	.....	180
3219.....	900,000,000	1,000,000,000	.....	262

<sup>1</sup> These eggs showed the development of at least 3-day embryos; in no case were the embryos living; therefore designated as blood rings.

Maximum average count..... 2,080,000,000

Minimum average count..... 350,000,000

Stiles and Bates, as a result of their investigations, arrive at the following tentative conclusions: Under normal conditions, strictly fresh eggs contain few, if any, bacteria. Strictly fresh, and commercially fresh, frozen eggs, held in storage for more than one year, showed little variation in their bacterial content during this period. *B. coli* was absent in the strictly fresh and commercially fresh samples.

**Effect of Dehydration on Bacteria.**—De Bord (*J. Agric. Res.*, 1925, 31, 155) has reported the results of a study of the effect of dehydration on the bacterial flora of eggs. The total number of viable bacteria in dehydrated egg varies with the quality of the raw egg but is, in general, greater in that dried in a vacuum drum than



**"ROTTEN EGGS"<sup>1</sup> FROZEN AND EXAMINED AT INTERVALS FROM  
MAR. 16, 1910, TO MAR. 29, 1911**  
(Four dozen eggs received by the trade, discards from candling)

Sample number	Organisms per c.c.		<i>B. coli</i> per c.c.	Days in storage
	At 25°	At 37°		
2303.....	720,000,000	1,400,000,000	100,000,000	0
2312.....	8,000,000,000	3,000,000,000	.....	7
2380.....	1,200,000,000	1,000,000,000	.....	15
2399.....	600,000,000	300,000,000	.....	21
2458.....	900,000,000	300,000,000	.....	46
2559.....	600,000,000	400,000,000	.....	49
2586.....	800,000,000	900,000,000	.....	56
2624.....	700,000,000	600,000,000	.....	69
2646.....	400,000,000	200,000,000	.....	79
2773.....	900,000,000	800,000,000	.....	155
2910.....	500,000,000	300,000,000	.....	176
2972.....	500,000,000	400,000,000	.....	220
3118.....	500,000,000	200,000,000	10,000,000	253
3218.....	600,000,000	300,000,000	.....	291
3372.....	600,000,000	600,000,000	.....	378

Maximum average count ..... 5,500,000,000

Minimum average count ..... 300,000,000

<sup>1</sup> Includes all classes of "rots" except "black rots" not used for tanning.

in that prepared by the spray process. By this process the total counts vary from 350 in the case of a good egg to over a million in "spots." These numbers are reckoned on a basis of 1 grm. of the liquid egg. In eggs dried on a vacuum drum the numbers vary from 45,000 to over 2 millions. The numbers depend not only on the initial count, but also on the time and temperature of storage. In good eggs after 10 months' storage at 37° and at 20° there was a diminution, whereas in bad samples there was sometimes an increase and sometimes a decrease. The characteristic smell of doubtful egg is almost lost in the drying process.

**Bacteria in Frozen Eggs.**—Verge and Grasset (*Compt. rend.*, 1928, 186, 718) have examined Chinese eggs imported into France and have shown that the frozen eggs compared advantageously with eggs in the shell as to number of colonies developing on incubation, but when left for 24 hours at laboratory temperature before incubation, the number of colonies per grm. were: white of egg on gelatin at 20°, 360,000; on gelose at 37°, 35,000; yolk, 3,600,000 and 3,500,000; and whole egg, 4,000,000 and 2,000,000, respectively. Besides bacteria which had lost all pathogenic power, others of the paratyphoid and coli groups were isolated, so that such frozen eggs should be used only in products that will be subjected to heat.

The same authors (*Rec. Med. Vet.*, 1929, 114, 657; *Rev. hyg. med. prev.*, 52, 239) have shown that eggs which have been frozen decompose very rapidly upon thawing, and should be used within a few hours. The eggs must be constantly kept solidly frozen, or deteriorative chemical and bacteriological changes occur. Inspection at the source is the best method of ensuring a high quality frozen product.

### FISH

Fish contain, in general, the same constituents as the higher vertebrates, but differ from them in containing more collagen and less extractives. The flesh of fish is for most species white, but some, as the salmon family, contain distinctive red colouring matters belonging to the lipochromes (fat pigments). The fat of fish is very liable to oxidation, and this accounts, to a large extent, for the fact that fish develops a strong and disagreeable flavour, unless consumed soon after death or unless preserved by sufficiently low temperatures (freezing). Great quantities of fish are held in frozen condition, but the temperature required is lower than for mammalian meat, in consequence of the readiness of oxidation of the fat.

**Composition of Flesh of Fish.**—Some of the earliest work on the composition of the flesh of fish is that of W. O. Atwater (*Amer. Chem. J.*, 1887, 9, 421) who reported the results of his analyses of a large number of fish caught in American waters. The accompanying table gives some of his results.

COMPOSITION OF AMERICAN FISH—ATWATER

Fish	No. of samples contributing to average	Water %	Nitrogenous matters %	Fat %	Ash %
Herring.....	2	74.6	14.5	9.0	1.78
Mackerel.....	8	71.2	19.4	8.0	1.36
Halibut.....	3	75.2	18.5	5.2	1.06
Conger-eel.....	2	71.4	18.5	9.1	1.00
Salmon.....	8	64.3	21.6	12.7	1.39
Cod.....	6	82.2	16.2	0.33	1.36
Plaice.....	2	78.3	18.7	1.9	1.01
Sole.....	1	86.1	11.9	0.25	1.22
Carp.....	1	76.9	21.86	1.1	1.33

In a subsequent paper (*ibid.*, 10, 1) Atwater gives the following data respecting the nature and proportions of nitrogenised matters, etc., in the water-free flesh of various fishes:

Fish	Coagulable albumin in cold-water extract %	Non-coagulable matter in cold-water extract %	Gelatinoids extracted by hot water %	Insoluble proteins %	Phosphoric acid %
Herring.....	5.23	4.51	9.46	.....	1.77
Mackerel.....	7.27	8.61	5.74	47.37	2.11
Halibut.....	0.42	7.04	12.89	28.14	1.81
Pike.....	6.95	9.55	10.20	56.71	2.21
Haddock.....	7.89	6.18	16.36	65.06	2.49

Atwater has also compared the composition of the ash yielded by the ignition of the flesh of the haddock and the pike, as typical of salt-water and fresh-water fishes, respectively.

Fish	Ash in dry flesh	Percentage composition of ash						
		K <sub>2</sub> O	Na <sub>2</sub> O	CaO	MgO	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	Cl
Haddock.....	11.26	13.84	36.51	3.39	1.90	13.70	0.31	38.11
Pike.....	6.13	23.92	20.45	7.38	3.81	38.16	2.50	4.74

From these figures the presence is apparent of a considerable proportion of sodium chloride in the flesh of salt-water fish; but the flesh of the pike also shows a much larger proportion of sodium salt than is found in the flesh of ruminants.

The following analyses of cooked fish are selected from a number published by K. I. Williams (*J. Chem. Soc.*, 1897, 71, 649).

The fish was prepared just as it would be served at table, being first cleaned and then boiled in water of 26 degrees of hardness (chiefly due to calcium carbonate). The salt cod and herrings were previously soaked in cold water for several hours, while the sardines were well washed in both boiling and cold distilled water, to remove as much of the surface-oil as possible. When cold, all the bones, head, and such portion of the skin as would not be eaten were removed and carefully weighed, crushed in a mortar, boiled in distilled water, and the liquid siphoned off and evaporated over a water-bath. The residue, when constant in weight, was taken as gelatin.

## COMPOSITION OF COOKED FISH—WILLIAMS

Name of fish	Date	Portion analyzed	As served at table			
			Waste bones etc.	Gelatin	Water	Nu- trients
Herrings.....	February	Whole	11.74	0.63	52.99	34.54
Salt herrings.....	January	Flesh	.....	.....	46.03	53.97
Sprats.....	November	Whole	17.90	0.90	61.50	19.70
Sardines.....	March	Whole	4.91	.....	42.17	52.92
Salmon.....	July	Section	5.99	0.53	61.06	32.02
Trout.....	May	Whole	8.23	0.55	67.12	24.10
Eels.....	October	Heads removed	11.66	1.09	53.29	33.96
Mackerel.....	April	Whole	10.51	0.25	65.21	24.03
Cod.....	January	Section	15.99	0.43	63.78	19.79
Salt cod.....	February	Section	6.13	0.33	67.68	25.86
Haddock.....	January	Whole	35.10	0.80	46.46	17.64
Whiting.....	January	Whole	25.10	0.86	61.29	16.35
Turbot.....	February	Anterior and head	31.20	0.59	53.09	15.12
Halibut.....	May	Section	6.84	0.03	69.35	23.78
Plaice.....	December	Flesh	.....	.....	79.86	20.14
Soles.....	March	Whole	22.02	0.74	61.18	16.06
Lemon soles.....	January	Whole	26.17	1.42	56.56	15.85
Oysters.....	March	Shell contents	.....	.....	77.71	22.29

The additional data shown on p. 578 were obtained by the analysis of the same samples of cooked fish.

The proteins in these analyses were estimated by multiplying the nitrogen, determined by the soda-lime method, by 6.25.

For the estimation of the reducing substances, the fat was first removed by benzene, and the residue digested with 100 c.c. of water and 10 c.c. of hydrochloric acid (sp. gr. 1.125) in a flask connected with a reflux condenser. The whole was heated as strongly as possible over a water-bath for 3 hours, the liquid filtered, treated with basic lead acetate, and the sulphur dioxide passed through the filtered liquid. The solution was again filtered, concentrated at 100°, and a little washed alumina added until it was no longer dissolved. The filtered liquid was then evaporated to dryness at 100°, the residue treated with boiling alcohol, the liquid filtered, and the alcohol distilled off. The residue was dissolved in water, the solution boiled with animal charcoal and a few drops of milk of lime, filtered, and the filtrate titrated with Fehling's solution.

The proportion of reducing substances shown in the foregoing analyses by Miss Williams of the flesh of fish is, in most cases, remark-

COMPOSITION OF DRIED SUBSTANCE OF COOKED FISH—  
WILLIAMS

Name of fish	Water in flesh of fish %	Analysis of dried substance						
		Ash %	Nitro- gen %	Phos- phorus %	Fat; or ether extract %	Pro- teins %	Reducing sub- stances reckoned as glucose %	Nitro- gen pen- toxi- de %
Herrings.....	60.54	5.56	11.11	0.91	25.25	67.07	.....	0.66
Salt herrings.....	46.03	10.69	7.12	0.89	21.90	38.88	17.59	1.64
Sprats.....	75.77	6.42	9.26	1.17	27.37	57.94	9.88	.....
Sardines.....	44.35	12.03	8.54	0.97	33.49	55.44	.....	.....
Salmon.....	65.32	4.94	10.70	0.51	29.43	56.65	14.89	0.46
Trout.....	73.58	6.60	11.96	1.13	8.84	80.00	4.68	.....
Eels.....	61.08	2.11	7.36	0.42	44.68	42.88	8.91	.....
Mackerel.....	73.13	4.07	10.46	0.85	25.73	62.32	13.93	0.33
Cod.....	76.32	3.31	15.30	0.62	1.15	91.55	6.67	0.63
Salt cod.....	72.35	14.26	12.41	0.29	0.94	76.06	7.14	0.31
Haddock.....	72.37	3.28	13.11	0.53	1.29	79.57	13.15	0.43
Whiting.....	78.78	1.92	13.28	0.73	1.86	79.55	17.54	.....
Turbot.....	77.84	2.41	13.76	0.57	4.75	84.71	11.81	.....
Halibut.....	74.46	4.11	13.32	0.67	15.81	79.67	.....	.....
Plaice.....	76.86	4.06	13.02	0.71	9.84	75.16	11.56	2.78
Soles.....	79.20	3.47	14.00	0.52	1.71	86.71	11.87	.....
Lemon soles.....	78.11	4.42	11.04	0.54	12.96	69.88	14.80	.....
Oysters.....	77.71	12.16	11.85	0.49	7.77	65.42	18.32	.....

ably large. As the method of estimation involved treatment with hydrochloric acid for some hours at the boiling-point of water, it seems probable that the reducing substances did not pre-exist as such, but were the products of the hydrolysis of bodies of the glyco-protein class. These compounds have been observed by Hammarsten (*Z. physiol. Chem.*, 19, 19; *J. Chem. Soc.*, 1894, 1, 310), Pavy (*Physiology of the Carbohydrates*, 1894), and others to result from action of hot dilute acids on protein matters. The conjecture receives support from the fact that the sum of the ash, fat, proteins, reducing substances, and nitrogen pentoxide is in some cases materially in excess of 100.00.

The presence of notable quantities of nitrates in the flesh of fish is remarkable. The analyses are not in accordance with the popular belief that the proportion of phosphorus is materially in excess of that present in the (dry) flesh of terrestrial animals.

Atwater and Bryant (*U. S. Dept. Agr. Off. Expt. Sta. Bull.* 28, rev., 1906) have collected much information of the composition of fish. The accompanying tables are taken from their report:

## COMPOSITION OF FISH—ATWATER AND BRYANT

Food materials	Number of analyses	Refuse	Water	Protein		Fat	Total carbohydrates	Ash	Fuel value per pound
				N X 6.25	By difference				
<b>FISH, FRESH.</b>		%	%	%	%	%	%	%	Cals.
Bass, striped, whole:									
Edible portion—									
Average.....	6	.....	77.7	18.6	18.3	2.8	.....	1.2	465
As purchased—									
Average.....	5	55.0	35.1	8.4	8.3	1.1	.....	.5	200
Bass, striped, entrails removed, as purchased.	1	51.2	37.4	8.8	8.7	2.2	.....	.5	255
Blackfish, whole:									
Edible portion—									
Average.....	4	.....	79.1	18.7	18.5	1.3	.....	1.1	405
As purchased—									
Average.....	2	60.2	31.4	7.4	7.3	.7	.....	.4	165
Blackfish, entrails removed, as purchased:									
Average.....	2	55.7	35.0	8.4	8.3	.5	.....	.5	175
Bluefish, entrails removed:									
Edible portion.....	1	.....	78.5	19.4	19.0	1.2	.....	1.3	410
As purchased.....	1	48.6	40.3	10.0	9.8	.6	.....	.7	210
Buffalo fish, entrails removed:									
Edible portion.....	1	.....	78.6	19.0	17.9	2.3	.....	1.2	430
As purchased.....	1	52.5	37.3	8.5	8.5	1.1	.....	.6	205
Butterfish, whole:									
Edible portion.....	1	.....	75.0	18.0	17.8	11.0	.....	1.2	800
As purchased.....	1	42.8	40.1	10.3	10.2	6.3	.....	.6	460
Catfish:									
Edible portion.....	1	.....	64.1	14.4	14.4	20.6	.....	.9	1,135
As purchased.....	1	19.4	51.7	11.6	11.6	16.6	.....	.7	915
Ciscoe, whole:									
Edible portion—									
Average.....	3	.....	74.0	18.5	18.1	6.8	.....	1.1	610
As purchased.....	1	42.7	43.6	11.1	11.0	2.0	.....	.7	290
Ciscoe, entrails removed, as purchased:									
Average.....	2	10.1	65.6	16.3	15.9	7.5	.....	.9	620
Cod, whole:									
Edible portion—									
Average.....	5	.....	82.6	16.5	15.8	.4	.....	1.2	325
As purchased—									
Average.....	2	52.5	38.7	8.4	8.0	.2	.....	.6	165
Cod, dressed, as purchased:									
Average.....	3	29.9	58.5	11.1	10.6	.2	.....	.2	215
Cod, sections, edible portion:									
Average.....	3	.....	82.5	16.7	16.3	.3	.....	.9	325
Cod, steaks:									
Edible portion.....	1	.....	79.7	18.7	18.6	.5	.....	1.2	370
As purchased.....	1	9.2	72.4	17.0	16.9	.5	.....	1.0	335
Cusk, entrails removed:									
Edible portion.....	1	.....	82.0	17.0	16.9	.2	.....	.9	325
As purchased.....	1	40.3	49.0	10.1	10.1	.1	.....	.5	190
Eels, salt water, head, skin, and entrails removed:									
Edible portion—									
Average.....	2	.....	71.6	18.6	18.3	9.1	.....	1.0	730
As purchased—									
Average.....	2	20.2	57.2	14.8	14.6	7.2	.....	.8	580
Flounder, whole:									
Edible portion—									
Average.....	3	.....	84.2	14.2	13.9	.6	.....	1.3	290
As purchased—									
Average.....	2	61.5	32.6	5.4	5.1	.3	.....	.5	115
Flounder, entrails removed, as purchased.	1	57.0	35.8	6.4	6.3	.3	.....	.6	130
Haddock, entrails removed:									
Edible portion—									
Average.....	4	.....	81.7	17.2	16.8	.3	.....	1.2	335
As purchased—									
Average.....	4	51.0	40.0	8.4	8.2	.2	.....	.6	165

## COMPOSITION OF FISH—ATWATER AND BRYANT.—(Continued)

Food materials	Number of analyses	Refuse	Water	Protein		Fat	Total carbohydrates	Ash	Fuel value per pound
				N X 6.25	By difference				
<b>FISH, FRESH—Continued.</b>		%	%	%	%	%	%	%	Cal.
Hake, entrails removed:									
Edible portion.....	1	.....	83.1	15.4	15.2	.7	.....	1.0	315
As purchased.....	1	52.5	39.5	7.3	7.2	.3	.....	.5	150
Halibut, steaks or sections:									
Edible portion.....	3	.....	75.4	18.6	18.4	5.2	.....	1.0	565
Average.....	3	17.7	61.9	15.3	15.1	4.4	.....	.9	470
Herring, whole:									
Edible portion.....	2	.....	72.5	19.5	18.9	7.1	.....	1.5	660
As purchased.....	2	42.6	41.7	11.2	10.9	3.9	.....	.9	375
Average.....	2	.....	72.5	19.5	18.9	7.1	.....	1.5	660
Kingfish, whole:									
Edible portion.....	1	.....	79.2	18.9	18.7	.9	.....	1.2	390
As purchased.....	1	56.6	34.4	8.2	8.1	.4	.....	.5	170
Lamprey, whole:									
Edible portion.....	1	.....	71.1	15.0	14.9	13.3	.....	.7	840
As purchased.....	1	45.8	38.5	8.1	8.1	7.2	.....	.4	455
Mackerel, whole:									
Edible portion.....	6	.....	73.4	18.7	18.3	7.1	.....	1.2	645
Average.....	5	44.7	40.4	10.2	10.0	4.2	.....	.7	365
As purchased.....	1	40.7	43.7	11.6	11.4	3.5	.....	.7	365
Mackerel, entrails removed, as purchased.	1	40.7	43.7	11.6	11.4	3.5	.....	.7	365
Mullet, whole:									
Edible portion.....	1	.....	74.9	19.5	19.3	4.6	.....	1.2	555
As purchased.....	1	57.9	31.5	8.2	8.1	2.0	.....	.5	235
Muskellunge, whole:									
Edible portion.....	1	.....	76.3	20.2	19.6	2.5	.....	1.6	480
As purchased.....	1	49.2	38.7	10.7	10.0	1.3	.....	.8	245
Perch, white, whole:									
Edible portion.....	2	.....	75.7	19.3	19.1	4.0	.....	1.2	530
Average.....	2	.....	75.7	19.3	19.1	4.0	.....	1.2	530
As purchased.....	2	62.5	28.4	7.3	7.2	1.5	.....	.4	200
Perch, pike (wall-eyed pike):									
Edible portion.....	1	.....	79.7	18.6	18.4	.5	.....	1.4	365
As purchased.....	1	57.3	34.0	7.9	7.9	.2	.....	.6	155
Perch, yellow, whole:									
Edible portion.....	2	.....	79.3	18.7	18.7	.8	.....	1.2	380
Average.....	1	62.7	30.0	6.6	6.7	.2	.....	.4	130
As purchased.....	1	35.1	50.7	12.8	12.6	.7	.....	.9	205
Perch, yellow, dressed, as purchased.	1	35.1	50.7	12.8	12.6	.7	.....	.9	205
Pickarel, pike, whole:									
Edible portion.....	3	.....	79.8	18.7	18.6	.5	.....	1.1	370
Average.....	3	.....	79.8	18.7	18.6	.5	.....	1.1	370
As purchased.....	2	47.1	42.2	9.9	9.9	.2	.....	.6	190
Average.....	2	47.1	42.2	9.9	9.9	.2	.....	.6	190
Pickarel, pike, entrails removed, as purchased.	1	42.7	45.7	10.7	10.7	.3	.....	.6	210
Pike, gray, whole:									
Edible portion.....	1	.....	80.8	17.9	17.3	.8	.....	1.1	365
As purchased.....	1	63.2	29.7	6.6	6.4	.3	.....	.4	135
Pollock, dressed:									
Edible portion.....	1	.....	76.0	21.6	21.7	.8	.....	1.5	435
As purchased.....	1	28.5	54.3	15.4	15.5	.6	.....	1.1	310
Pompano, whole:									
Edible portion.....	2	.....	72.8	18.8	18.7	7.5	.....	1.0	665
Average.....	2	.....	72.8	18.8	18.7	7.5	.....	1.0	665
As purchased.....	2	45.5	39.5	10.3	10.2	4.3	.....	.5	375
Average.....	2	45.5	39.5	10.3	10.2	4.3	.....	.5	375

## COMPOSITION OF FISH—ATWATER AND BRYANT.—(Continued)

Food materials	Number of analyses	Refuse	Water	Protein		Fat	Total carbohydrates	Ash	Fuel value per pound
				N X 6.25	By difference				
<b>FISH, FRESH—Continued.</b>		%	%	%	%	%	%	%	Cals.
Porgy, whole:									
Edible portion—									
Average.....	3	.....	75.0	18.6	18.5	5.1	.....	1.4	560
As purchased—									
Average.....	3	60.0	29.9	7.4	7.4	2.1	.....	.6	225
Red grouper, entrails removed:									
Edible portion—									
Average.....	2	.....	79.5	19.3	18.8	.6	.....	1.1	385
As purchased—									
Average.....	2	55.9	35.0	8.5	8.4	2	.....	.5	165
Red snapper, whole:									
Edible portion—									
Average.....	3	.....	78.5	19.7	19.2	1.0	.....	1.3	410
As purchased—									
Average.....	2	46.1	42.0	10.8	10.6	.6	.....	.7	225
Red snapper, entrails and gills removed, as purchased.	1	45.3	43.7	10.6	10.0	.3	.....	.7	210
Salmon, whole:									
Edible portion—									
Average.....	6	.....	64.6	22.0	21.2	12.8	.....	1.4	950
As purchased—									
Average.....	4	34.9	40.9	15.3	14.4	8.9	.....	.9	660
Salmon, entrails removed, as purchased.									
Average.....	2	29.5	48.1	13.8	13.5	8.1	.....	.8	600
Salmon, landlocked, whole, spent:									
Edible portion—									
Average.....	4	.....	77.7	17.8	17.8	3.3	.....	1.2	470
As purchased—									
Average.....	4	45.5	42.3	9.7	9.8	1.8	.....	.6	255
Salmon, California, anterior sections:									
Edible portion—									
Average.....	2	.....	63.6	17.8	17.5	17.8	.....	1.1	1,080
As purchased.....	1	10.3	57.9	16.7	16.1	14.8	.....	.9	935
Shad, whole:									
Edible portion—									
Average.....	7	.....	70.6	18.8	18.6	9.5	.....	1.3	750
As purchased—									
Average.....	7	50.1	35.2	9.4	9.2	4.8	.....	.7	380
Shad, roe, as purchased.....	1	.....	71.2	20.9	.....	3.8	2.6	1.5	600
Sheepshead, whole:									
Edible portion—									
Average.....	2	.....	75.6	20.1	19.5	3.7	.....	1.2	530
As purchased.....	1	66.0	26.9	6.6	6.4	.2	.....	.5	130
Sheepshead, entrails removed, as purchased.	1	56.6	31.2	9.0	8.8	2.9	.....	.5	290
Skate, lobe of body:									
Edible portion.....	1	.....	82.2	18.2	15.3	1.4	.....	1.1	400
As purchased.....	1	51.0	40.2	8.9	7.5	.7	.....	.6	195
Smelt, whole:									
Edible portion—									
Average.....	2	.....	79.2	17.6	17.3	1.8	.....	1.7	405
As purchased—									
Average.....	2	41.9	46.1	10.1	10.0	1.0	.....	1.0	230
Spanish mackerel, whole:									
Edible portion.....	1	.....	68.1	21.5	21.0	9.4	.....	1.5	795
As purchased.....	1	34.6	44.5	14.1	13.7	6.2	.....	1.0	525
Sturgeon, anterior sections:									
Edible portion.....	1	.....	78.7	18.1	18.0	1.9	.....	1.4	415
As purchased.....	1	14.4	67.4	15.1	15.4	1.6	.....	1.3	350
Tomcod, whole:									
Edible portion.....	1	.....	81.5	17.2	17.1	.4	.....	1.0	335



## COMPOSITION OF FISH—ATWATER AND BRYANT.—(Continued)

Food materials	Number of analyses	Refuse	Water	Protein		Fat	Total carbohydrates	Ash	Fuel value per pound
				N X 6.25	By difference				
<b>FISH, FRESH—Continued.</b>		%	%	%	%	%	%	%	Cals.
Tomcod, whole:									
As purchased.....	1	59.9	32.7	6.9	6.8	.2	....	.4	133
Trout, brook, whole:									
Edible portion—									
Average.....	3	.....	77.8	19.2	18.9	2.1	....	1.2	445
As purchased—									
Average.....	3	48.1	40.4	9.9	9.8	1.1	....	.6	30
Trout, salmon or lake:									
Edible portion—									
Average.....	2	.....	70.8	17.8	17.7	10.3	....	1.2	765
As purchased—									
Average.....	2	43.5	36.6	9.1	9.2	5.1	....	.6	385
Turbot:									
Edible portion.....	1	.....	71.4	14.8	12.9	14.4	....	1.3	885
As purchased.....	1	47.7	37.3	7.7	6.8	7.5	....	.7	460
Weakfish, whole:									
Edible portion.....	1	.....	79.0	17.8	17.4	2.4	....	1.2	430
As purchased.....	1	51.9	38.0	8.6	8.4	1.1	....	.6	205
Whitefish, whole:									
Edible portion.....	1	.....	69.8	22.9	22.1	6.5	....	1.6	700
As purchased.....	1	53.5	32.5	10.6	10.3	3.0	....	.7	377
<b>FISH, COOKED.</b>									
Bluefish, cooked, edible portion.	1	.....	68.2	25.9	26.1	4.5	....	1.2	670
Spanish mackerel, broiled:									
Edible portion.....	1	.....	68.9	23.7	23.2	6.5	....	1.4	715
As purchased.....	1	7.9	63.5	21.8	21.4	5.9	....	1.3	655
<b>FISH, PRESERVED AND CANNED<sup>1</sup></b>									
Cod, salt: <sup>2</sup>									
Edible portion—									
Average.....	2	.....	53.5	25.4	21.5	.3	....	<sup>3</sup> 24.7	410
As purchased—									
Average.....	2	24.9	40.2	19.0	16.0	.4	....	18.5	315

PHOSPHORIC ANHYDRIDE, SULPHURIC ANHYDRIDE AND CHLORINE IN SAMPLES OF PRESERVED AND CANNED FISH<sup>1</sup>

Kind of fish	Phosphoric anhydride		Sulphuric anhydride		Chlorine	
	Number of estimations	Average	Number of estimations	Average	Number of estimations	Average
Cod, salt. <sup>2</sup> .....	2	%	2	%	2	%
Cod, salt, boneless.....	1	.36	1	.68	1	11.92
Halibut, smoked.....	1	.47	1	.44	1	8.66
Herring, smoked.....	1	.84	1	1.24	1	7.21
Mackerel, salt <sup>2</sup> .....	1	.35	1	.61	.....	.....
Salmon, canned.....	1	.61	1	.44	.....	.....

<sup>1</sup> A considerable number of determinations of phosphorus, sulphur, and chlorine have been made in the flesh of preserved and canned fish. These are recorded in the following table in terms of phosphoric anhydride ( $P_2O_5$ ), sulphuric anhydride ( $SO_3$ ), and chlorine (Cl), and in percentages of the total weight of "edible portion" or flesh.

<sup>2</sup> It is observable that in salt cod the proportion of protein by difference is much smaller than by factor. The former value is apparently more nearly correct, and has been used in estimating the fuel value per pound.

<sup>3</sup> Two samples averaged 23 % common salt.

Williams (*Chem. News*, 1911, 104, 271) gives some very complete analyses of 25 varieties of English fish.

Clark and Almy (*J. Biol. Chem.*, 1918, 33, 483) report the analysis of twenty common food fishes and show a seasonal variation largely due to changes in fat content. The accompanying table is taken from their report.

COMPOSITION OF FISH—CLARK AND ALMY

Variety of fish	Season	Total solids %	Fat %	Ash %	Total nitrogen %	Cold-water soluble nitrogen %	Coagulable nitrogen %	Hot-water soluble nitrogen %	Ammonia %
Blackfish.....		20.00	0.15	1.40	2.93	0.874	0.532	0.241	0.0172
Bonita.....		26.66	1.46	1.71	3.82	1.030	0.389	0.088	0.0251
Cod.....		18.65	0.09	1.23	2.95	0.950	0.530	0.330	0.0170
Herring.....		22.90	2.41	1.66	3.03	1.028	0.516	0.234	0.0177
Kingfish.....		24.60	5.24	1.39	2.83	0.838	0.425	0.172	0.0162
Ling.....		18.30	0.12	1.15	2.69	1.030	0.650	0.240	0.0180
Porgy.....		23.39	2.59	1.37	3.02	1.001	0.620	0.221	0.0165
Tilfish.....		19.66	0.51	1.35	2.80	0.770	0.380	0.220	0.0130
Silver hake.....		18.86	1.41	1.22	2.60	1.025	0.638	0.211	0.0140
Bluefish.....	S <sup>1</sup>	23.83	1.54	1.16	3.36	0.760	0.320	0.071	0.0185
Bluefish.....	F <sup>1</sup>	29.04	8.10	1.11	3.26	0.730	0.360	0.085	0.0180
Butterfish.....	S	25.66	5.96	1.49	2.89	0.862	0.498	0.020	0.0162
Butterfish.....	F	30.01	13.52	1.40	2.92	0.850	0.430	0.021	0.0180
Carp sucker.....	S	22.80	2.10	1.20	2.98	1.151	0.787	0.206	0.0154
Carp sucker.....	F	24.79	4.17	1.20	3.17	1.078	0.748	0.210	0.0170
Croaker.....	S	20.77	1.25	1.18	2.85	0.813	0.447	0.141	0.0140
Croaker.....	F	24.26	3.23	1.37	.....	0.880	0.410	0.130	0.0230
Flounder.....	S	17.54	0.20	1.17	2.56	0.888	0.477	0.117	0.0143
Flounder.....	F	21.59	0.37	1.34	2.54	0.740	0.330	0.200	0.0190
Haddock.....	S	18.32	0.15	1.11	2.33	0.748	0.343	0.147	0.0220
Haddock.....	F	20.83	0.09	1.01	2.59	0.800	0.520	0.150	0.0220
Striped bass.....	S	25.70	3.58	1.26	3.21	1.153	0.719	0.102	0.0170
Striped bass.....	F	19.83	2.98	1.26	3.07	1.050	0.650	.....	0.0166
Sea bass.....	S	22.02	1.61	1.23	2.98	0.967	0.532	0.106	0.0159
Sea bass.....	F	19.44	1.60	1.09	.....	0.970	0.700	.....	0.0220
Spanish mackerel.....	S	33.01	12.59	1.20	3.13	0.846	0.458	0.055	0.0185
Spanish mackerel.....	F	35.70	16.24	1.11	3.09	0.890	0.570	0.102	0.0240
Weakfish.....	S	21.41	2.34	1.25	2.83	1.118	0.851	0.203	0.0134
Weakfish.....	F	19.35	0.52	1.20	.....	0.820	0.500	0.290	0.0150
Shad, male.....	.....	35.32	14.43	1.34	3.18	1.112	0.621	0.074	0.0160
Shad, female.....	Apr.	34.17	13.93	1.40	3.00	1.147	0.685	0.063	0.0182
Shad, female.....	May	26.00	5.87	1.29	2.91	0.980	0.549	0.058	0.0191
Shad, female spent.....	June	23.38	2.95	1.53	2.98	0.975	0.549	0.182	0.0178

<sup>1</sup> S = Spring. F = Fall.

Dill (*J. Biol. Chem.*, 1921, 48, 73) has made a study of the composition of the edible portion of mackerel (*Scomber japonicus*). The following are his general results secured from 27 lots, mostly individuals:

	Fattest %	Thinnest %	Maximum %	Minimum %
Solids.....	41.05	24.35	41.05	24.35
Fat.....	20.32	0.41	20.32	0.41
Ash.....	1.04	1.41	1.04	1.56
Protein.....	20.44	23.06	20.31	24.31

A second set of results secured on 21 lots, with 1 to 10 fish in a lot, is as follows:

	Fattest %	Thinnest %	Maximum %	Minimum %
Solids.....	38.80	23.38	38.80	23.38
Fat.....	18.12	0.28	18.12	0.28
Ash.....	1.31	1.47	1.24	1.50
Protein.....	20.44	22.19	19.75	23.57

Some of Dill's results on varieties of tuna and bonito are shown in the following table:

Variety		Solids %	Fat %	Ash %	Protein %
Albacore ( <i>Germo alalunga</i> ).....	5 fat	38.49	12.76	1.35	24.06
Albacore ( <i>Germo alalunga</i> ).....	20 others	32.19	5.50	1.32	25.97
Blue fin tuna ( <i>Thunnus thynnus</i> )...	10	30.04	5.22	1.37	24.79
Yellow fin tuna ( <i>Germo macrepterus</i> )	30	28.51	3.05	1.40	24.67
Striped tuna ( <i>Gymnosarda pelamis</i> )	10	34.06	7.37	1.30	25.41
Bonita ( <i>Sarda chilensis</i> ).....	6	26.26	1.21	1.47	24.81
Bonita ( <i>Sarda chilensis</i> ).....	1 fat	41.08	19.21	1.34	20.44

The chemical composition of the edible portion of sardines has been reported by Dill (*J. Biol. Chem.*, 1921, 48, 93). His results are shown herewith:

Grade	Weight gm.	Solids %	Fat %	Protein %
Quarters.....	15- 40	23.73	1.76	20.62
Halves.....	48- 70	24.79	3.56	20.13
Small ovals.....	80-120	27.51	6.46	19.81
Large ovals.....	140-260	31.87	11.76	18.84
Low fat <sup>1</sup> .....	165	21.66	0.89	19.37

<sup>1</sup> This sample had 1.60 % ash.

Shostrom, Clough, and Clark (*J. Ind. Eng. Chem.*, 1924, 16, 283) have made a study of canned salmon, and report the accompanying figures for the composition of the bone-free fish.

#### COMPOSITION OF CANNED SALMON—SHOSTROM, CLOUGH, AND CLARK

Variety	No. of cans	Bone %	Water %	Fat %	Salt- free ash %	Protein %	Calories per lb.
Chinook.....	44	1.12	65.56	11.59	1.16	19.60	853
Sockeye.....	126	1.96	67.19	8.58	1.32	21.04	753
Chinook.....	216	1.78	63.98	13.41	1.21	19.51	928
Coho.....	99	1.88	67.49	8.49	1.24	21.08	750
Pink.....	90	2.32	70.05	6.20	1.31	20.56	644
Chum.....	108	1.87	70.58	5.15	1.28	21.48	615
Steelhead trout.....	20	1.67	57.70	20.09	1.21	19.55	1,211
Atlantic salmon.....	6	1.14	64.30	12.49	1.22	21.14	920
Average:							
Special packs.....	639	1.96	67.91	8.37	1.27	20.73	738
Commercial packs.....	167	2.33	66.95	10.52	1.05	20.13	768

Gibbs (*Food Manufacture*, July 1927, p. 70) has given the following composition for Manx summer herrings collected during the season of 1916.

Month	Condition	Water %	Fat %	Protein %	Ash %	Calories per lb.
May.....	Empty	75.0	2.5	21.1	2.3	1,100
June.....	Filling	66.1	11.4	18.6	2.0	1,806
July.....	Filling	55.8	21.6	18.4	2.3	2,762
August.....	Half full	48.4	31.5	16.5	2.3	3,608
September.....	Full	51.9	25.2	17.3	2.6	3,050

The nutritive value of fish and shell fish is treated in *Appendix X* to the *Report of the U. S. Commissioner of Fisheries* for 1925. The following figures are taken from that report.

## COMPOSITION OF CANNED FISH

	Solids %	Fat %	Protein %	Ash %
Mackerel.....	31.82	8.68	19.63	1.30
Salmon, Chinook.....	38.12	15.70	20.18	1.32
Sardines in oil, French.....	43.63	12.71	24.87	5.61 <sup>1</sup>
Sardines in oil, American.....	47.85	25.52	19.17	3.84 <sup>1</sup>
Sardines in tomato sauce, American.....	32.33	5.55	18.08	3.94 <sup>1</sup>
Tuna in oil.....	46.40	19.60	25.40	1.40

<sup>1</sup> Includes salt.

## COMPOSITION OF SALTED, SMOKED, PICKLED, AND DEHYDRATED FISH

	Solids %	Fat %	Protein %	Ash %
Boneless salt cod.....	45.6	0.3	26.3	23.2 <sup>1</sup>
Desiccated cod.....	88.4	4.9	72.0	5.2
Smoked haddock.....	27.4	0.2	23.3	1.5
Smoked halibut.....	50.6	15.0	20.8	2.1
Pickled herring.....	57.7 <sup>2</sup>	19.7	25.0	4.0
Smoked herring.....	65.5	15.8	36.9	1.5
Salt mackerel.....	56.1	25.1	18.6	2.6

<sup>1</sup> Includes salt.

<sup>2</sup> Contains 4.04 % acids, sugars, etc.

The iodine content of fish and sea foods is treated in the following reports: *Department of Commerce, Bureau of Fisheries, Appendix I and Appendix VI* to the *Report of the U. S. Commissioner of Fisheries* for 1924; *University of Washington Publications in Fisheries* 1, No. 6, pp. 109-140, February, 1926.

**Iron in Fish.**—Peterson and Elvehjem (*J. Biol. Chem.*, 1928, 78, 215), in connection with their work on the iron content of foods, have reported the figures on p. 587 for iron in fish.

**Arsenic in Fish.**—The Swedish Commission on Arsenical Poisoning showed that the amount of arsenic normally present in fish is much greater than has hitherto been suspected, the higher results being attributable to the greater accuracy of the process of digestion with nitric and sulphuric acids introduced by Bang and Ramberg. An

## IRON CONTENT OF FISH—PETERSON AND ELVEHJEM

	Moisture %	Iron %
Bass.....	77.0	0.00026
Bluefish.....	76.7	0.00060
Catfish.....	80.0	0.00036
Cod.....	81.7	0.00034
Flounder.....	80.0	0.00073
Haddock.....	78.8	0.00042
Halibut.....	67.3	0.00093
Herring.....	77.6	0.00059
Lake trout.....	79.0	0.00078
Lobster.....	81.1	0.00044
Mackerel.....	77.6	0.00075
Oysters.....	87.5	0.00314
Perch.....	80.4	0.00048
Pickrel.....	72.5	0.00080
Pike.....	80.2	0.00034
Red snapper.....	79.2	0.00040
Salmon.....	75.7	0.00083
Shad.....	69.8	0.00053
Shrimp.....	70.4	0.00267
Whitefish.....	79.8	0.00042

outline of the method finally adopted by the Swedish Commission is given in "The Analyst" (1925, 50, 6) and its accuracy has been confirmed by H. E. Cox (*Analyst*, 1925, 50, 1), who concludes that it gives satisfactory results for amounts of not less than 0.01 mg., but that for smaller amounts the Ramberg method of destruction, as recommended by the Swedish Commission (*loc. cit.*), followed by the Marsh-Berzelius method of determination, is the most accurate.

Bang, in his work for the Swedish Commission, found as much as 4 parts per million of arsenic in some fish, and in a Report of the Ministry of Agriculture and Fisheries (*Analyst*, 1924, 49, 484) it is mentioned that as much as 3.7 parts per million of arsenious oxide was found in certain Whitstable oysters.

There is convincing evidence that arsenic is rapidly excreted by the system after the eating of fish. Thus L. Brahme (*Arsen in Blut*, Stockholm, 1923) found arsenic in the blood within an hour after a meal of fish, and Cox (*loc. cit.*) has confirmed the results of Bang, who found that the arsenic was rapidly excreted in the urine, appearing within 24 hours.

The following table shows the amounts of arsenic (as  $\text{As}_2\text{O}_3$ ) per 100 grm. of the edible part of the respective fish found by Cox, and for comparison the results obtained by Bang are also tabulated:

ARSENIC AS  $\text{As}_2\text{O}_3$  PER 100 GRM. OF FISH

Fish	British Arsenic Committee's method, mg.	Swedish Commission's method (Ramberg), mg.	Amounts found by Bang, mg.
<i>(British)</i>			
Whiting.....	0.01	0.04	0.01-0.33
Plaice.....	0.08	0.14	.....
Plaice.....	.....	0.25	.....
Plaice.....	.....	0.30	.....
Sole.....	0.02	0.03	.....
Hake.....	0.02	0.03	.....
Herring.....	0.01	0.03	0.04-0.08
Cod.....	0.01	0.05	0.08-0.20
John Dory.....	0.01	.....	.....
Haddock.....	0.01	0.06	.....
Brill.....	0.01	0.03	.....
Mackerel.....	0.02	0.05	.....
Halibut.....	0.00	0.03	.....
Turbot.....	0.02	0.05	0.06-0.18
Perch.....	.....	.....	0.08
<i>(Swedish)</i>			
Mackerel.....	0.01	0.01	.....
Herring.....	0.01	0.03	.....
Haddock.....	0.01	0.02	.....
Plaice.....	0.03	0.05	.....
Plaice.....	0.05	0.09	.....
Sole.....	0.01	0.01	.....
Cod.....	0.02	0.04	.....

Cox directs attention to the high proportion of arsenic present in the plaice, as compared with the sole, and suggests that the explanation is to be found in the fact that the former feed largely on bivalves, which have been found to contain considerable amounts of arsenic, whereas the principal food of the latter consists largely of worms and starfish. Other fish appear to derive their arsenic from marine algae, which have been shown to contain up to 0.7 mg. of arsenic per kilo (Tassilly and Leroide, *Bull. Soc. Chim.*, 1911, 9, 63).

Chapman (*Analyst*, 1926, 51, 548) found even greater amounts of arsenic in shell fish and crustaceans. Thus four samples of English oysters contained from 3 to 10 parts per million; Portuguese oysters (4 samples) from 33 to 70 parts per million; escallops (8 samples) 36 to 85 parts per million; mussels (6 samples) 36 to 119 parts; cockles (6 samples), 20 to 40 parts; whelks (6 samples), 20

to 40 parts; and periwinkles (6 samples) from 20 to 40 parts per million of the wet substance.

The edible portions of different lobsters contained from 36 to 40 parts of arsenic per million, and it was found that boiling had no material effect upon the amount.

*Prawns* (*Nephrops norvegicus*) contained from 38 to 100 parts per million in the edible portion, and 20 to 70 parts in the internal organs, whilst the shells contained 5 parts per million in one specimen and 7 parts in another.

*Shrimps*.—The eight specimens examined contained 17 to 40 parts of arsenic per million of wet substance.

*Crabs*.—Six specimens contained from 36 to 70 parts per million.

Chapman concludes that the arsenic is present in the lobster in the form of a more or less complex organic compound or compounds which are soluble in alcohol and in water. Apparently, arsenic in this form has less toxic effect than arsenious oxide, but Chapman suggests that the unpleasant consequences which sometimes occur after eating lobsters or shell fish may possibly be connected with the presence of these arsenic compounds.

**Fish Roe and Caviare.**—J. König gives the following analyses illustrating the percentage composition of caviare:

	No. of samples	Water %	Nitroge- nous substances %	Fat %	Nitrogen- free extract %	Ash %	Com- mon salt %
Caviare.....	5	43.89	30.79	15.66	1.67	8.09	6.02
Paionsnäja.....	1	30.89	40.33	18.90	.....	9.88	.....
Fish-roe cheese.	1	19.38	34.81	28.87	(6.33)	10.61	.....

Gobley gives the following as the composition of the eggs of the carp (compare hens' eggs, p. 540): water, 64.08; paravitellin, 14.06; fat, 2.57; cholesterol, 0.27; lecithin, 3.04; cerebrin, 0.21; membranous substance, 14.53; extractive matters, 0.39; coloring matters, 0.03; and salts, 0.82%.

König and Groszfeld (*Biochem. Z.*, 1913, **54**, 338, 351) found fish roe to contain xanthine, hypoxanthine, creatinine, taurine, *l*-tyrosine, glycocoll, thymine, proteins soluble and insoluble in water, and fat. The proteins are rich in sulphur and phosphorus and do not yield protamines. The fat is characterised by a high lecithin



content (up to 59%) and also contains from 3.9 to 14% of cholesterol. For detailed analyses see above paper and also *Z. Nahr. Genussm.*, 1914, 27, 502.

Helen Chernoruzkii (*Z. physiol. Chem.*, 1912, 80, 194) found 1.2 grm. of nucleic acid in 100 grm. of freshly dried and alcohol-ether extracted herring eggs.

Kodama (*Arch. Hyg.*, 1913, 78, 247) has shown that by means of the precipitin, anaphylaxis (active and passive) and complement-binding reactions, caviare can be differentiated from other fish spawn, such as carp, red eye, bream, tench, salmon, herring, and trout. By means of the precipitin reaction the fish-roe protein can be clearly differentiated from the flesh protein of the same animal.

Dinslage (*Z. Nahr. Genussm.*, 1913, 26, 200) found a sample of caviare preserved with urotropin (hexamethylene-tetramine), which he identified by means of Rimini's method as modified by Arnold and Mentzel (*Z. Nahr. Genussm.*, 1902, 5, 353).

**Anchovy Butter.**—Behre and Frerichs (*Z. Nahr. Genussm.*, 1912, 24, 676) claim that true anchovy butter is characterised by a lower fat content and lower iodine value and refraction of the extracted fat than herring butter, or a mixture of the two. In general, anchovy butter containing less than 10% of fat contains no foreign fish; that containing about 15% of fat must be regarded as suspicious if the iodine value exceeds 60 and the refraction 50. The addition of foreign fish cannot be detected in mixtures containing equal parts of fish and butter.

**Cured Fish.**—Large quantities of fish are cured by salting, especially cod, haddock, cusk, hake and pollock (U. S. A.) and mackerel. Sardines and other small fat fish are packed in oil. Enormous quantities of fish, especially salmon, are canned commercially. The composition of some of the most important cured and canned fish are reported above (pp. 575, 582, 585).

**Forms of Nitrogen in Fish Muscle.**—A few investigators have studied the forms of nitrogen in fish. Wilson (*J. Biol. Chem.*, 1914, 17, 385; 1914, 18, 17) has studied the partition of the nitrogen in extracts of the muscles of the lamprey, limulus, squid, clam, scallop, and periwinkle. Betaine was isolated from the scallop, periwinkle, and lamprey, and creatine from the lamprey.

Okuda (8th Int. Cong. Appl. Chem., 1912, 18, 275) obtained the following results in grm. per 100 grm. of dry substance:

	Creatine %	Creatinine %
Bonito.....	2.01	0.48
Tunny fish.....	1.80	0.23
Salmon.....	1.53	0.18
Snapper.....	3.33	0.31
Carp.....	2.02	0.37
Shark.....	3.24	0.66
Lobster, crab, clam, and cuttlefish.....	trace	trace

Yoshimura and Kanai (*Z. physiol. Chem.*, 1913, 88, 346) found dried codfish to yield per kilogram 1.4 grm. of creatinine, 0.44 grm. of betaine hydrochloride, 0.70 grm. of methylguanidine picrate, 13 grm. of taurine, 0.50 grm. of alanine, traces of glutamic acid, and no creatine or choline.

Hunter (*J. Biol. Chem.*, 1929, 81, 513) has reported the creatine content of the muscles and other tissues of fishes. Some of his results are shown below.

#### CREATINE OF TAIL MUSCLES OF FISH—HUNTER

Variety	No. of specimens	Creatine %
Dog fish.....	4	0.600
Skate.....	3	0.481
Rat fish.....	2	0.549
Herring.....	3	0.740
Coho salmon.....	2	0.636
Perch.....	4	0.669
Blue perch.....	2	0.613
Red rock cod.....	1	0.494
Rock cod.....	1	0.531
Rock cod.....	1	0.527
Rock trout.....	1	0.615
Ling cod.....	3	0.609
Sculpin.....	1	0.609
Bull head.....	2	0.564
Flat fish.....	4	0.609

These figures are in contrast with those obtained by Hunter on the mixed flesh of rabbits (0.525) and on muscles of most other animals, usually less than 0.500.

**Glycogen in Fish.**—Schöndorff and Wachholder (*Pflüger's Arch. Physiol.*, 1914, 157, 147) found the glycogen content of fish muscle to range from none to 0.68%.

**Frozen Fish.**—Clark and Almy (*J. Ind. Eng. Chem.*, 1920, 12, 656) have made a study of frozen fish in storage for short or long

periods of time. The water, fat, and ash were determined by the A. O. A. C. methods, the ammonia and amine nitrogen by the Steel-Gies modified method (*J. Biol. Chem.*, 1918, 33, 486). The accompanying table shows the results. No detectable changes

EFFECT OF FREEZING AND STORAGE ON THE COMPOSITION OF  
FOOD FISHES, EDIBLE PORTION—CLARK AND ALMY

	Storage period, months	Total solids %	Fat %	Protein %	Ash %	Calories per pound
Fresh eviscerated weakfish.....	.....	24.41	5.15	18.75	1.18	566
Fresh eviscerated weakfish.....	.....	21.30	1.76	18.50	1.24	418
Frozen eviscerated weakfish glazed.....	2	22.39	1.91	18.69	0.96	428
Frozen eviscerated weakfish glazed.....	4	23.35	2.47	19.56	1.10	468
Frozen eviscerated weakfish glazed.....	8	23.08	1.99	19.50	1.18	446
Frozen eviscerated weakfish glazed.....	13	24.86	2.82	20.00	1.16	491
Frozen eviscerated weakfish glazed.....	25	24.84	2.87	19.80	1.10	489
Fresh eviscerated bluefish.....	.....	23.98	1.13	21.50	1.34	447
Fresh eviscerated bluefish.....	.....	24.86	1.82	20.68	1.32	461
Frozen eviscerated bluefish glazed	4	25.80	3.33	20.87	0.99	529
Frozen eviscerated bluefish glazed	5	26.02	3.19	20.31	1.14	512
Frozen eviscerated bluefish glazed	8	25.68	2.02	22.30	1.27	500
Frozen eviscerated bluefish glazed	12	25.26	1.85	21.06	1.22	470
Frozen eviscerated bluefish glazed	16	25.71	1.81	21.06	1.23	468
Frozen eviscerated bluefish glazed	16	24.33	0.63	22.30	1.26	442
Frozen eviscerated bluefish glazed	27.5	26.22	1.33	22.69	1.23	478

were found even after 27 months of storage in the frozen and glazed condition. These investigators also determined the different nitrogenous substances obtained by the water-extract method.

The acidity in the fat of the fish increased with storage from 5.5-12.4 in fresh weakfish and 3.9-16.0 in fresh bluefish fat up to 20-26 in stored weakfish and 40-41 in stored bluefish.

**Fresh and Stale Fish.**—There are various methods of distinguishing fresh fish from stale fish by means of physical properties. Almy (*J. Amer. Chem. Soc.*, 1927, 49, 2540) has made a study of free and combined cystine with special reference to certain effects produced by heating fish flesh. Twenty grm. of flesh are placed compactly in a 20 × 150 mm. test tube of soft glass, which is sealed in a blast lamp and heated in a constant temperature bath at 120° for 45 minutes. The tube is inverted in a beaker containing 25 c.c. of 6

COMPOSITION OF WATER EXTRACT OF FROZEN FISH—CLARK AND ALMY

	Storage period, months	Solids %	Ash %	Acidity	Total nitrogen %	Coagulable nitrogen %	Tannin + salt ppt. nitrogen %	Protease nitrogen %	Amino nitrogen %	Ammonia and amine nitrogen %	Peptones, bases, etc. (by difference) %
Fresh eviscerated weakfish.....	.....	7.49	1.04	15.4	0.86	0.55	0.040	0.010	0.054	0.014	0.233
Frozen eviscerated weakfish.....	.....	7.28	1.11	15.0	0.77	0.50	0.033	0.017	0.054	0.015	0.288
Frozen eviscerated weakfish glazed.....	2	6.23	0.97	18.5	0.83	0.49	0.035	0.010	0.082	0.013	0.235
Frozen eviscerated weakfish glazed.....	4	4.10	1.05	15.1	0.84	0.52	0.040	0.015	0.078	0.015	0.220
Frozen eviscerated weakfish glazed.....	8	4.22	1.14	10.0	0.95	0.53	0.045	0.010	0.073	0.010	0.216
Frozen eviscerated weakfish glazed.....	13	7.28	0.97	10.0	0.81	0.49	0.034	0.018	0.060	0.017	0.224
Frozen eviscerated weakfish glazed.....	25	7.36	0.97	18.0	0.81	0.46	0.027	0.008	0.077	0.018	0.254
Fresh eviscerated bluefish.....	.....	7.16	1.24	35.4	0.93	0.48	0.045	0.034	0.102	0.017	0.281
Fresh eviscerated bluefish.....	.....	8.50	1.30	30.0	1.00	0.58	0.054	0.050	0.107	0.017	0.310
Frozen eviscerated bluefish.....	.....	4.54	1.14	30.0	0.77	0.42	0.055	0.044	0.113	0.016	0.175
Frozen eviscerated bluefish glazed.....	4	5.65	1.24	27.1	0.76	0.33	0.012	0.035	0.112	0.018	0.266
Frozen eviscerated bluefish glazed.....	5	5.49	1.17	28.0	0.82	0.44	0.056	0.034	0.103	0.021	0.220
Frozen eviscerated bluefish glazed.....	8	6.80	1.19	38.0	0.85	0.31	0.050	0.044	0.143	0.022	0.320
Frozen eviscerated bluefish glazed.....	12	6.27	1.15	30.1	0.79	0.30	0.041	0.028	0.112	0.024	0.310
Frozen eviscerated bluefish glazed.....	16	6.98	1.18	31.4	0.86	0.35	0.071	0.021	0.120	0.021	0.350
Frozen eviscerated bluefish glazed.....	16	6.98	1.18	31.4	0.86	0.35	0.071	0.021	0.120	0.021	0.350
Frozen eviscerated bluefish glazed.....	27.5	7.22	1.23	37.4	0.86	0.33	0.058	0.032	0.137	0.026	0.334

Acidity = c.c. 0.1 of N NaOH per 50 c.c. of extract

% zinc acetate solution, and the tip of the tube is broken off. The beaker and contents and tube are all placed in a vacuum desiccator, the evacuation of which draws the gas in the tube through the zinc acetate solution, thus trapping any hydrogen sulphide contained in the air space above the flesh. The tube is then carefully broken in the centre, and the contents are ground thoroughly in a mortar and washed into a cylinder for the determination of hydrogen sulphide by the methylene blue method (Almy, *J. Amer. Chem. Soc.*, 1925, 47, 1381). This method estimates both the gas which is combined as sulphide and that which exists free in the flesh. The term "hydrogen sulphide," as used in this paper, includes both forms.

Employing this method Almy has shown the effect of holding fish at room temperature to be as follows:

HYDROGEN SULPHIDE IN MMG. PER 100 GRAMS OF FLESH

	Fresh		24 hours		48 hours		72 hours	
	H <sub>2</sub> S	pH	H <sub>2</sub> S	pH	H <sub>2</sub> S	pH	H <sub>2</sub> S	pH
Weakfish.....	33	6.5	3,296	7.0	3,859	7.1	.....	7.3
Rockfish.....	34	6.0	59	6.3	1,809	6.5	2,922	6.6

Almy believes that the results indicate the possibilities of distinguishing fresh from stale fish by this means. Fresh flesh has the power of oxidising hydrogen sulphide, whilst stale flesh has lost this power. The work also shows that heating flesh above 100° C. causes a loss of hydrogen sulphide from the cystine, and perhaps from other sulphur compounds.

### Methods of Analysis of Fish

The methods already described for meat are entirely satisfactory for the analysis of fish. The vacuum oven (or vacuum desiccator) is especially necessary for making moisture estimations and for drying extracted oil. The ready oxidisability of the fats and the volatile nature of some of the hydrogen sulphide in the fish flesh demand low-temperature drying.

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